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References: 1. Panos, T. C.: South. M. J. 48:527 (May) 1955. 2. Fisher, D. A.; Hammond, G. D., and Pickering, D. E.: Am. J. Dis. Child. *90*:6 (July) 1955. 3. Van Wyk, J. J.: Quart. Rev. Pediat. *10*:212 (Nov.) 1955.

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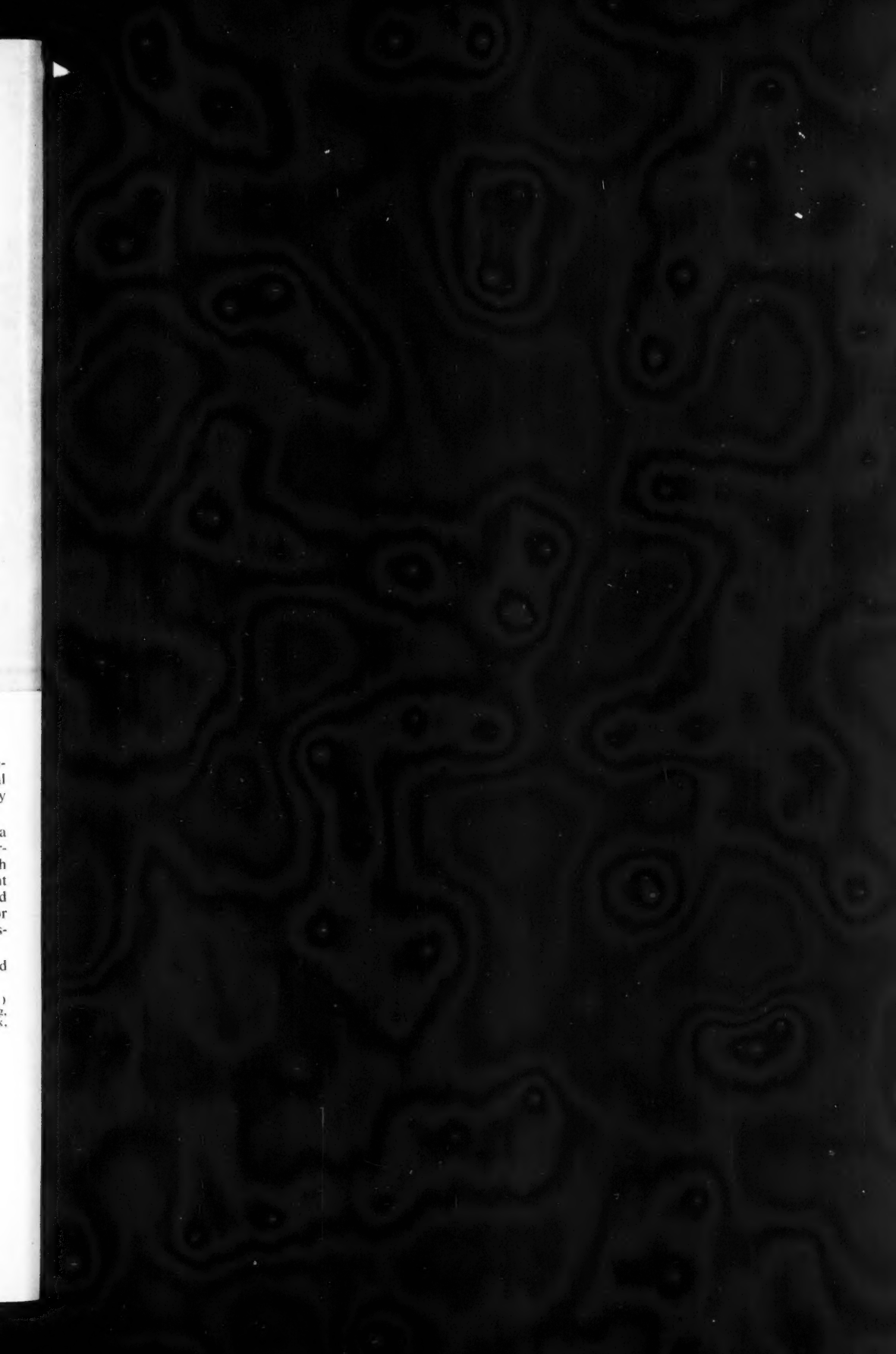

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In the article by Luft, R., and von Euler, U. S.: Effect of insulin hypoglycemia on urinary excretion of adrenaline and noradrenaline in man after hypophysectomy, *J. Clin. Endocrinol. & Metab.* **16**: 1017-1025 (Aug.) 1956, the excretion of adrenaline and of noradrenaline should be expressed in terms of mμg. per minute instead of μg. per minute.

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METABOLISM OF 4-C^{14}-CORTISOL IN MAN: BODY DISTRIBUTION AND RATES OF CONJUGATION*†

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THE dynamics of cortisol metabolism in normal men at levels ordinarily maintained by endogenous sources has been only partly explored. Hellman *et al.* (1) have recently presented data on the rate and routes of excretion of 4-C^{14}-cortisol in 2 patients with breast cancer, 1 with chronic lymphatic leukemia and 1 with multiple benign lipomata. Over 90 per cent of the radioactivity administered was excreted in the urine and feces in seventy-two hours. Little or no radioactivity was found in the exhaled carbon dioxide of these patients. In addition, the dynamic behavior of 4-C^{14}-

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† Part of data presented at the Annual Meeting of The Endocrine Society at Atlantic City, June 2–4, 1955 (abstr. *J. Clin. Endocrinol. & Metab.* **15**: 851, 1955).

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cortisol in an adrenalectomized oophorectomized patient was studied when the subject was untreated and when she was maintained on fairly large doses of intravenous cortisol. Peterson *et al.* (2) have studied 4-C^{14}-cortisol metabolism in normal subjects and found the hormone to have a half-life of seventy to ninety minutes in the blood, with metabolic pools of 1.3 to 2.4 mg.

The present paper describes some of the results obtained during an investigation of the rate of metabolism of 4-C^{14}-cortisol and its distribution in various tissues of the human body. Although no attempt was made to identify precisely the various metabolites, the proportion of radioactivity readily extractable by organic solvents and the amounts released by several types of hydrolyses were determined.

METHODS

Experimental subjects

Five young male adults, medical students or laboratory technicians, were selected as normal subjects.

For a study of biliary secretion of 4-C^{14}-cortisol, 2 patients (N and J) with cholecystitis associated with the presence of stones in the gallbladder were selected. The following liver function tests yielded normal results in both patients prior to operation: thymol turbidity, sulfobromophthalein (BSP), alkaline phosphatase, blood bilirubin and urinary urobilinogen. After cholecystectomy, a T-tube was inserted for drainage of bile, and each patient received an injection of 4-C^{14}-cortisol four days (Patient N) and five days (Patient J) after operation. The completeness of the collections of bile was enhanced by attaching a suction pump to the T-tube.

A study of the distribution of radioactivity in various tissues was carried out in 1 woman (Patient R), who received 4-C^{14}-cortisol one hour prior to cholecystectomy for cholecystitis. Cyclopropane and nitrous oxide were used for anesthesia. The tissue samples were removed at the time of operation.

Purity of 4-C^{14}-cortisol

4-C^{14}-cortisol was provided by the Endocrinology Study Section, Division of Research Grants, National Institutes of Health, Bethesda Maryland. Its specific activity was 1.467 millicuries per millimole. An accompanying note stated that 95 per cent of the radioactivity moved as cortisol when analyzed by paper chromatography (solvent systems: benzene-formamide and toluene-propylene glycol) at the Worcester Foundation. The material was also analyzed in our laboratory by paper chromatography using the Bush (3) system of benzene/methanol:water (55:45). To an aliquot of the radioactive material, nonradioactive cortisol was added. The mixture was chromatographed and the area of the paper strip corresponding to cortisol eluted; 90.2 per cent of the radioactivity of the aliquot was found in the eluate.

Mode of administration of 4-C^{14}-cortisol

Each batch of 4-C^{14}-cortisol was dissolved in redistilled ethanol so that 1 milliliter of the solution contained approximately 1 microcurie, and was kept in the refrigerator. An aliquot of this solution was assayed for radioactivity.

All solutions were prepared and injected by the same individual throughout the entire study. For intravenous administration, 1 ml. of 4-C^{14}-cortisol solution was carefully pipetted out, transferred into a sterile beaker at the time of the experiment, and diluted with 10 ml. of sterile physiologic saline solution. The mixture was aspirated into a syringe and injected intravenously over a period of one to two minutes. At the end of the injection a 1-ml. or 2-ml. sample of blood was drawn into the syringe and immediately reinjected in order to minimize the loss of radioactive material in the syringe. Syringe and needle were then washed with water and the beaker which contained the test dose was rinsed with ethanol. The various washings were pooled and their radioactivity was determined. In order to calculate the exact dose administered, this value was subtracted from the known radioactivity of the original aliquot.

All 4-C^{14}-cortisol injections were made between 2:26 and 3:20 P.M. This period of the day was chosen because it has been shown that at this time plasma 17-hydroxycorticosteroid levels do not vary so much as earlier in the day (4). Exception was made in the case of Patient R, who received the injection at 7:15 A.M., one hour prior to the beginning of the surgical procedure.

Mode of collection of samples of blood, urine, bile, feces and tissues.

Samples of 30–50 ml. of blood were collected in heparinized syringes 15, 30, 60, 120 and 240 minutes after the injection of the radioactive material (the time in the middle of the injection was taken as starting time). The blood samples were immediately centrifuged and the plasma separated from the red cells. Urine and bile samples were collected 2, 4, 8, 16, 24 and 48 hours following the injection. Feces were collected and pooled during the 48 hours following cortisol administration.

Various tissue samples were taken from Patient R at the time of operation—127 Gm. of fat from the abdomen 90 minutes after injection; 3.7 Gm. of abdominal muscle 120 minutes after injection; and 2.1 Gm. of liver 150 minutes after injection. Using the formula published by Kyle (5) for the calculation of the percentage of fat in the total body weight, it was estimated that Patient R had 18 Kg. of body fat. The amount of muscle in this patient was approximately 27 Kg., based upon the data of Magnus-Levy (6) as quoted by West and Todd (7). A value of 1,600 Gm. was taken for the total weight of the liver.

All samples were stored in the deep-freeze until the time of extraction or counting.

Method of extraction of the various specimens

Each plasma sample was extracted three times with an equal volume of freshly redistilled chloroform, in order to obtain the free steroids. A 100-ml. portion of absolute ethanol was then added to the plasma residue. After shaking and centrifuging, the liquid phase was decanted and the solid part was washed with 50 ml. of ethanol. The pooled ethanol extracts were evaporated to dryness. The residue was taken up with 20 ml. of distilled water, incubated at 47°C. with β-glucuronidase (approximately 750 units per ml. of plasma) at pH 4.6 (acetate buffer) for fifteen hours, and extracted with chloroform (40 ml. $\times$ 3) to obtain the steroids formerly present as conjugates of glucuronic acid. The pooled plasma residues from each patient were then subjected to a 24-hour continuous extraction with ether at pH 0.8 in order to obtain other easily hydrolyzable conjugates of the steroids. Following this procedure, the aqueous residue was boiled for twenty minutes with one-tenth its volume of concentrated HCl and extracted with chloroform to obtain steroids probably present as conjugates but not hydrolyzed by the preceding procedures.

The red blood cells were extracted three times with their volume of absolute ethanol in order to obtain free and conjugated steroids.

A 20-ml. aliquot of each urine and bile collection was extracted three times with an equal volume of chloroform in order to obtain the free steroids (Fraction I). A β-glucuronidase hydrolysis as described for plasma was then carried out (Fraction II), followed by a 24-hour continuous extraction with ether at pH 0.8 (Fraction III). A 3-ml. portion of concentrated hydrochloric acid was added to the aqueous residue, the mixture boiled for twenty minutes and then extracted three times with its volume of ether (Fraction IV).

Each 48-hour feces collection was extracted twice with 95 per cent ethanol (3,000 ml.) using a Waring blendor for mixing. The ethanol extracts were separated from feces by centrifugation.

The liver and muscle specimens were homogenized in 20 ml. of water and were then extracted with chloroform, followed by β-glucuronidase hydrolysis, continuous ether extraction at pH 0.8 and strong acid hydrolysis as described for urine. A portion of fat tissue weighing 21.2 Gm. was homogenized with approximately 50 ml. of hexane, made up to 100 ml. with hexane and extracted with 70 per cent ethanol (75 ml.×3). Water was added to the ethanol extract in order to obtain an approximately 50 per cent ethanolic solution, which was then extracted with chloroform (100 ml.×3). The chloroform extracts contained mainly the free steroids; and the 50 per cent ethanol, largely the conjugated steroids.

All the extracts were evaporated to dryness as soon as possible in a water bath at 40° C., using a stream of air.

Radioactivity determinations

All extracts of plasma, red blood cells, urine, bile, feces and tissues, as well as whole urine and bile, were assayed for radioactivity. Each assay was made in duplicate. The specimen was dissolved in an appropriate solvent, and an aliquot sufficiently large to furnish a significant count was deposited from a micropipette on the surface of an aluminum disc mounted on a slowly rotating turntable. Drying was hastened by a warm stream of air from an ordinary hair dryer. The weight of material on the aluminum discs was determined by differential weighings before and after plating, and a correction was made for self-absorption. The assays were made in a windowless flow-counter. The samples were counted for a period sufficiently long to obtain at least 80 per cent and in most cases 95 per cent accuracy.

RESULTS

The amount of radioactivity readily extractable from plasma by chloroform following the injection of 4-C^{14}-cortisol in the various experimental subjects is shown in Table 1, along with the amount of radioactivity extractable after β-glucuronidase hydrolysis. The results are expressed as a percentage of the administered dose per liter of plasma. Figure 1 shows the rate of change of radioactivity in the free and the glucuronide fractions of the plasmas from the 5 normal subjects. Only very small additional amounts of radioactivity were found after continuous ether extraction at pH 0.8 (less than 0.05 per cent of the dose per liter of plasma).

Our data show that the intravenously administered cortisol disappeared

TABLE 1. RADIOACTIVITY READILY EXTRACTABLE FROM PLASMA WITH CHLOROFORM, AND THAT EXTRACTABLE FOLLOWING β-GLUCURONIDASE HYDROLYSIS AT VARIOUS INTERVALS FOLLOWING 4-C^{14}-CORTISOL ADMINISTRATION (EXPRESSED AS PERCENTAGE OF THE ADMINISTERED DOSE PER LITER OF PLASMA)

Subjects	Age and sex	Body wt. (Kg.)	Injected dose (c/m)	After chloroform					After β-glucuronidase				
				15 min.	30 min.	60 min.	120 min.	240 min.	15 min.	30 min.	60 min.	120 min.	240 min.
Controls													
A	26 M		979,000	4.75	3.99	2.93	1.62	0.66	0.45	0.66	—	—	0.88
D	25 M	69.5	985,000	5.58	4.16	2.36	1.54	0.47	—	0.85	1.22	1.52	1.08
E	24 M	72.0	979,000	—	3.28	2.13	1.23	0.50	—	1.22	1.24	1.84	1.28
K	27 M	76.5	1,121,000	4.15	3.84	2.50	1.55	0.47	0.53	0.71	0.87	1.09	0.72
P	26 M	83.0	1,111,000	4.08	3.38	1.91	1.39	0.55	0.54	0.48	0.70	0.89	0.27
Biliary-fistula patients													
J	49 F	60.0	1,119,000	—	—	1.64	0.70	0.46	0.97	0.94	1.59	1.74	1.13
N	65 M	68.0	1,123,000	3.74	2.42	2.21	1.69	0.69	0.53	0.55	0.73	0.58	0.29
Surgical patient													
R	29 F	67.5	5,551,000	4.44	3.57	2.67	1.37	0.78	0.80	1.13	1.38	1.42	1.08

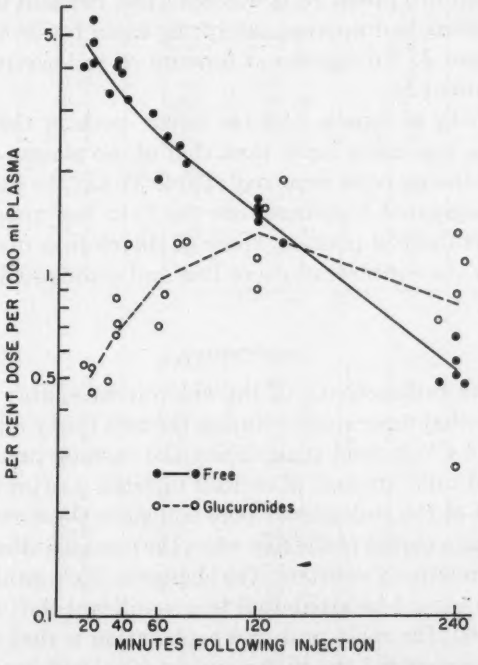


FIG. 1. Plasma radioactivity in 5 control subjects. The lines join the averages at the various times studied.

rapidly from the blood; fifteen to twenty minutes after the injection only 12 to 15 per cent of the dose was present as free steroid in the estimated total plasma volume. At that time 1.5 per cent was found in a conjugated form and less than 0.2 per cent in the total red cell mass. Since very little radioactivity had been excreted in the urine and bile at that time, approximately 85 per cent of the dose had been removed from the blood stream into the extracellular fluid and/or the tissues.

The cumulative urinary excretion of total radioactivity at various times after the injection of 4-C^{14}-cortisol is shown in Table 2. The table also shows the radioactivity extractable from urine without hydrolysis, following β-glucuronidase hydrolysis, during continuous ether extraction at pH 0.8, and after strong acidification and boiling. The results are expressed in per cent of the administered dose. Figure 2 shows the average cumulative urinary excretion of radioactivity by the normal subjects, and Figure 3 the average rate of urinary excretion of radioactivity by the same subjects.

Figure 4 shows the cumulative biliary excretion of radioactivity in the various fractions in Patient J (biliary fistula). The total radioactivity in bile was 5.0 per cent of the injected dose in this patient forty-eight hours after the injection. In Patient N, it was only 1.62 per cent of the dose, but the various fractions had approximately the same ratios of radioactivity as those of Patient J. No significant amount of radioactivity was found in the feces of patient N.

The radioactivity of muscle and fat tissue, both in the free and conjugated fractions, was much lower than that of the plasma at the approximate time these tissues were removed (Table 3). On the other hand, both the free and conjugated fractions from the liver had approximately the same activities as those of plasma. There is, therefore, a marked difference among tissues in the concentrations of free and conjugated steroids found in them.

DISCUSSION

Apparently the radioactivity of the chloroform-soluble steroids in the plasma fell somewhat more steeply during the first thirty minutes following the injection of 4-C^{14}-cortisol than during the ensuing period (one to four hours). Since the total amount of cortisol injected was probably not more than 10 per cent of the endogenous pool and since these experiments were carried out during a period of the day when the concentration of this steroid in the plasma is relatively constant, the change in the rate of disappearance from the plasma cannot be attributed to a significant shift in total concentration of cortisol. The most probable explanation is that the first thirty-minute period represented the time required for the radioactive molecules to come into equilibrium with the non-radioactive steroids in the body

TABLE 2. CUMULATIVE URINARY EXCRETION OF RADIOACTIVITY FOLLOWING INJECTION OF 4-C^{14}-CORTISOL (EXPRESSED AS PERCENTAGE OF THE ADMINISTERED DOSE)

Patients	Hrs. after injection	Total (% dose)	"Free" (no hydrolysis)	β-Glucuronidase	Ether extr. (continuous) at pH 0.8	Strong-acid hydrolysis +boiling	Unaccounted for
Normal subjects							
A	2	14.4	1.07	2.8	2.4	2.0	6.13
	4	33.3	1.67	13.2	4.5	3.8	10.13
	8	51.2	2.0	24.3	6.9	4.2	13.8
	14	69.6	2.27	34.6	9.2	4.9	18.6
	24	79.1	2.5	40.8	10.9	5.5	19.4
	48	87.4	2.61	44.5	12.0	—	—
D	2	14.4	0.38	10.7	2.0	0.6	0.72
	4	29.6	0.47	18.0	3.2	1.3	6.63
	8	49.1	0.59	32.0	5.2	2.5	8.8
	16	68.8	0.82	45.0	6.8	4.0	12.2
	24	76.0	0.87	50.0	7.7	4.5	12.9
	48	83.8	0.90	52.0	8.1	4.8	18.0
E	2	13.4	0.53	9.2	1.9		
	4	28.1	0.70	19.0	3.1		
	8	52.4	1.15	32.0	5.4		
	16	73.4	1.49	44.0	8.1		
	24	81.8	2.01	47.0	9.1		
	48	89.5	2.08	49.5	10.0		
K	2	16.4	0.50	8.1	1.8	1.0	5.0
	4	34.1	0.69	16.0	4.0	2.1	11.3
	9	62.4	1.44	32.8	7.2	4.6	16.9
	16	76.7	1.83	36.8	9.7	5.7	22.7
	31.5	89.2	2.61	39.2	10.8	6.8	29.8
	48	91.4	2.67	40.0	10.9	7.04	30.8
P	2	16.0	0.40	5.7	1.5	0.7	7.7
	4	33.2	0.77	16.1	2.9	1.8	11.7
	8	54.3	1.11	26.7	5.2	2.4	18.9
	16	73.3	1.36	34.9	8.2	3.5	25.3
	24	83.3	1.45	39.4	9.3	4.0	29.3
	48	88.3	1.47	40.4	9.7	4.2	33.0
Biliary-fistula patients							
J	2	11.4	0.50	4.7	1.3	1.2	3.7
	4	25.0	1.1	10.1	3.1	2.2	8.5
	8	48.3	1.5	18.6	6.5	2.4	19.3
	12	62.0	2.1	23.7	8.0	4.0	24.2
	24	73.8	2.6	29.4	9.9	5.4	26.5
	48	81.4	2.8	33.8	10.0	6.1	28.7
N	2	13.8	0.7	5.1	1.7	0.52	5.8
	4	33.3	1.5	11.7	5.1	1.6	11.4
	8	62.4	2.8	25.2	8.1	2.9	18.4
	12	68.4	3.0	30.3	8.7	3.6	22.8
	24	72.5	3.1	33.0	—	4.0	—
	48	76.8	3.2	33.5	—	4.3	—
Surgical patient							
R	15	65.4	4.0	29.0	9.2	4.5	18.7
	24	71.8	4.2	32.0	10.0	5.4	20.2
	48	75.2	4.3	33.0	10.4	5.6	21.9

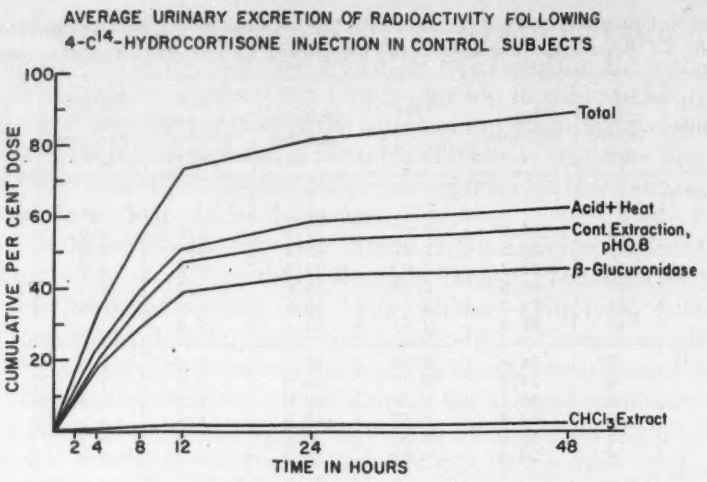
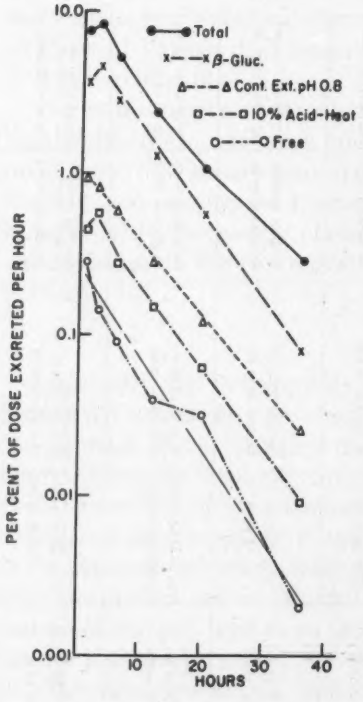


FIG. 2. Average cumulative urinary excretion of radioactivity following 4-C^{14}-cortisol injection in 5 control subjects.



compartments. Since the logarithms of the values between one and four hours fall approximately on a straight line, it seems probable that during this time, equilibrium existed between the free steroid pool and the plasma. The half-life of eighty minutes, then, is the time required for exchange of half of the free steroids. Recent paper chromatographic studies of the free fraction have shown that the radioactivity eluted from the area of the chromatograms corresponding to cortisol represents only 30 to 50 per cent of the total radioactivity of the chloroform-soluble steroids (8, 9). It would seem reasonable to assume that the rest of the radioactivity is due to metabolites of cortisol which are not

FIG. 3. Rates of excretion of radioactivity in the fractions obtained from urine of 5 control subjects.

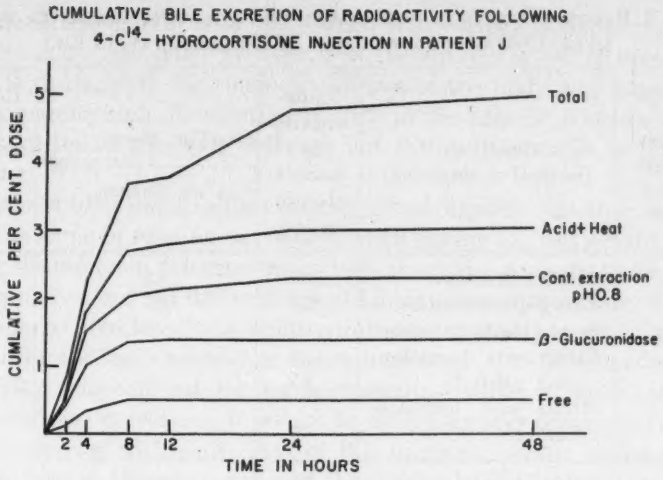


FIG. 4. Cumulative biliary excretion of radioactivity following 4-C^{14}-cortisol injection in Patient J.

conjugated already, such as tetrahydrocortisol, tetrahydrocortisone and the recently isolated cortol and cortolone (10). Under these conditions, it is difficult at present to determine the total metabolic pool of free cortisol.

Steroids conjugated with glucuronic acid in plasma reach equilibrium with the free steroids in about two hours—the time when the former has reached its peak. This is what one would expect, either if the conjugates were direct products of the free compound or if the first step in the conversion were the rate-limiting reaction. Since the major products conjugated with glucuronic acid in plasma are 3α-hydroxyl compounds (9, 11), there are probably two reductive steps involved in addition to the conjugation. Thus, it seems probable that the reduction to the dihydro derivative, the first reaction, is the rate-limiting step. This is borne out by observations made with infusions of nonradioactive cortisol, tetrahydrocortisol, cortisone, dihydrocortisone and tetrahydrocortisone (12). With any of the reduced compounds, the removal from the plasma was more rapid than with cortisol or cortisone and the conjugated compounds appeared more promptly in the blood.

At the time of collection of the various tissues from Patient R, and considering a total plasma volume of 2.5 liters, 8.9 per cent of the radioactivity administered was present in the total blood volume. From the data on normal and bile-fistula subjects, Patient R would have excreted 18.0 per cent of the dose in the urine and 2.7 per cent in the bile. If all the radioactivity not found in blood, urine and bile (namely 70.4 per cent of the dose) were in the extracellular fluid, the latter concentration would be al-

TABLE 3. RADIOACTIVITY IN VARIOUS TISSUES OF PATIENT R. (ESTIMATED WEIGHTS: LIVER, 1,600 GM.; BODY MUSCLE, 27 KG.; BODY FAT, 18 KG.)

Amount of tissue studied	Fraction (method of obtaining)	Radioactivity in sample studied (c/m)	$\frac{\text{c/m/Gm. tissue}}{\text{c/m/ml. plasma}} \times 100$*	Estimated radioactivity in total organ or tissue (c/m)
Liver (2.1 Gm.)	$CHCl_3$ extraction	133	83	102,000
	First β-glucuronidase	224	88	171,000
	Second β-glucuronidase	0	—	—
	Ether extr. (continuous), pH 0.8	45	—	34,000
	Strong acid hydrol.+ boiling	0	—	—
Muscle (3.7 Gm.)	$CHCl_3$ extraction	50	18	365,000
	β-glucuronidase	31	7	226,000
	Ether extr. (continuous), pH 0.8	0	—	—
	Strong acid hydrol.+ boiling	0	—	—
Fat (21.2 Gm.)	Ethanol extraction	23	1.4	195,000
	$CHCl_3$ extraction	63	2.5	535,000

* Although the various tissue samples were taken at different times after the injection (fat, 90 min.; muscle, 120 min.; liver, 150 min.), their radioactivity is compared with that of plasma 135 min. after the administration of steroid.

most double that found in plasma (assuming a volume of 10 liters for the extracellular fluid of Patient R). On the other hand, if the concentration of radioactivity in extracellular fluid were the same as that of plasma, approximately 34 per cent of the dose would have to be in the cells. The amount of radioactivity found in muscle and fat was small. This amount was probably not significantly different from that attributable to the blood and extracellular fluid which would be present, assuming the same radioactivity in both fluids. It seems, therefore, that little if any 4-C^{14}-cortisol penetrated into the muscle and fat cells.

In contrast, the liver had about the same concentration of both free and conjugated steroids as the plasma. Apparently the liver cells are very permeable to the hormone and its metabolites. The amount of radioactivity in the liver cell, however, would not alone account for the 34 per cent of the dose which would have to be in the cells, if the concentration of radioactivity in extracellular fluid were the same as that of plasma. This might mean that other tissues not sampled are penetrable and may even concentrate

the hormone. Dougherty *et al.* (13) found that cortisol is concentrated in fibroblasts and mast cells; perhaps it is the concentration of cortisol in these cells throughout the connective tissue of the body which accounts for the apparent high distribution volume in the face of evidence that a large proportion of the body cells are not readily permeable to the hormone.

The urine studies indicate that the rate of conjugation remained approximately the same in relation to concentration during the forty-eight hours following the injection. The curves for both the major conjugated fractions (β-glucuronidase and pH 0.8 hydrolysis) fall at a logarithmic rate; that of the fraction set free by strong-acid hydrolysis is probably similar, but the values during the last twenty-four hours were too low to be accurate. The radioactivity unaccounted for by compounds soluble in lipid solvents after the various hydrolyses, however, became a greater proportion of the total radioactivity with time. Part of this unaccounted-for radioactivity was due to loss in the preparation of the different fractions, but it seems unlikely that this is the full explanation. It would not account for the consistent increase in the proportion of this fraction. It seems probable, therefore, that a portion of the hormone was converted into material which no longer had the usual characteristics of steroid conjugates. This material apparently was slowly formed in the tissues and never reached equilibrium with the radioactive pool during the 48-hour period.

These experiments indicate that the metabolic products of cortisol excreted in the urine are probably conjugated with more than one substance. Although the glucuronides comprise the largest fraction, the fact that continuous extraction at pH 0.8 after glucuronidase incubation, even when the latter was repeated, released about one-quarter as much additional radioactivitiy suggests that sulphate or some other easily hydrolyzable group also reacts but at a slower rate. The amounts found after strong-acid hydrolysis may represent some other form of conjugates; on the other hand, they may simply be additional amounts of easily hydrolyzable material, since extraction at pH 0.8 for a longer period than twenty-four hours was not tried. Using a β-glucuronidase hydrolysis at pH 5 for five days at 37° C., followed by a 48-hour continuous extraction with ether, Gallagher *et al.* (14) were able to obtain a proportion of administered radioactivity approximately equal to the sum of our fractions released by β-glucuronidase and by continuous extraction with ether at pH 0.8. Fukushima *et al.* (10) recently characterized four new metabolites of adrenocortical hormones: cortol, cortolone and their isomers. These steroids appeared to be more water-soluble than tetrahydrocortisol, and consequently continuous extraction might be a more effective method for these steroids when set free by enzymatic hydrolysis. On the other hand, one might wonder if heating a solution acidified to pH 5 at 37° C. for five days and then holding at this

pH during a subsequent 48-hour continuous extraction might not free steroids which were conjugated with compounds other than glucuronic acid. Lieberman *et al.* (15) have shown the difficulty of differential hydrolytic procedures.

The amount of radioactivity in the urine which was extractable with chloroform before hydrolysis (so-called "free") was much less during the first four hours than would be expected on the basis of renal filtration of the free compound from the plasma (0.79 per cent of the dose compared with 43 per cent expected on the basis of 120 ml. of filtrate per minute). Several factors might explain this finding. Cortisone, according to Eik-Nes *et al.* (16), tends to bind loosely with serum albumin. Undoubtedly, cortisol has a similar tendency to bind with the protein, and hence this would decrease the amount of the filtrable chloroform-soluble steroid. Renal tubular reabsorption may also reduce the amount of free steroid excreted, and conjugation may occur in the kidney even though the liver is the major site of this reaction (17). In any case, as with other steroid hormones (18), the loss of the free steroid by way of the kidney is very small.

The appearance of radioactivity in the bile of the 2 patients with bile fistulas was essentially complete forty-eight hours after the injection of 4-C^{14}-cortisol and totaled 1.62 per cent and 5.0 per cent of the administered dose. No significant amount of radioactivity was found in the feces of Patient N, demonstrating the completeness of bile collection in this case. The amount of radioactivity found in the bile was approximately equivalent to the amount of radioactivity found in the feces of individuals without bile fistulas, as demonstrated by other workers (2, 19). This suggests that, at most, only partial reabsorption takes place in the gastro-intestinal tract. Since the amounts secreted in the bile are so small and variable, however, no conclusions can be drawn regarding the proportion reabsorbed. If the liver is the main site of degradation of cortisol and conjugation of its metabolites (17), it appears that the greater proportion of the conjugated compounds is not excreted in the bile and reabsorbed from the gastro-intestinal tract, but is released directly into the blood stream by way of the hepatic vein. It is interesting to note that there may be species differences here, as in the case of estrogens, androgens and progesterone (20, 21, 22). Wyngaarden *et al.* (23) found a large excretion of radioactivity in the bile following administration of 4-C^{14}-cortisol to rats and guinea pigs. The same authors demonstrated a reabsorption and recirculation of the labeled biliary metabolites in these two species.

Urinary excretion by the patients (N and J) with bile fistulas was somewhat lower than that of normal subjects. The same finding was observed in Patient R, and might represent the effect of surgical procedures on cortisol metabolism. The high plasma levels which are observed following severe surgical procedures have been shown by Tyler *et al.* (24) to be associated

with a temporary decrease in hepatic function reflected in a decreased rate of steroid conjugation. That such a factor accounts in part for the slower elimination of the isotopic molecules in Patients J, N and R is supported by the relatively high amount of isotopic material in the chloroform-soluble fraction of the urine and the reduction in activity in the steroids set free by β-glucuronidase.

SUMMARY

The distribution of radioactivity in blood, bile and urine after injection of 4-C^{14}-cortisol was studied as a function of time in normal subjects, patients with bile fistulas, and in a patient undergoing a major surgical operation. The activity of the chloroform-soluble ("free") steroid and the material which became chloroform-soluble after hydrolysis with β-glucuronidase, after continuous extraction at pH 0.8 and after boiling with 10 per cent hydrochloric acid, was determined in the samples.

The radioactivity in the "free" fraction from the plasma, after a preliminary period of thirty to sixty minutes, decreased at a logarithmic rate, with a mean half-life of eighty minutes. The isotopic activity of the glucuronide fraction in plasma reached a maximum at approximately two hours. At this time, it equalled that of the free fraction.

Analyses of muscle, fat and liver samples during the period when equilibrium had apparently been established in the plasma indicated that little or no hormone had penetrated the first two tissues, but that the liver cells were freely permeable to both the hormone and its metabolites.

Only a small amount of the hormone was excreted in the bile. This was taken as evidence that the metabolites of the hormones were realeased from the liver into the general circulation largely by way of the hepatic vein. The data also suggested that little or no reabsorption from the gastro-intestinal tract takes place and that the entero-hepatic circulation of this steroid plays little role in its metabolism.

The curves of the radioactivity of the urinary glucuronides and the conjugates easily hydrolyzable with acid were logarithmic from the second to the forty-eighth hour, indicating a constant rate of conjugation in relation to concentration. There was a gradually increasing proportion of non-hydrolyzable, water-soluble radioactivity. It is thought that this may represent some more completely metabolized form which is slowly released in the body.

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ESTROGENIC LEYDIG-CELL TUMOR WITH MULTIPLE METASTASES IN A DOG. THE PROBLEM OF BISEXUAL HORMONE PRODUCTION BY GONADAL CELLS

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TESTICULAR tumors in dogs are of interest because of their high incidence (1–5) and the light they shed on the hormonal activity of the various cell types occurring in the testes of man. Proof has been amassed within recent years that the Sertoli cells and their respective tumors can produce estrogenic hormone (6–9).

The outstanding feature described in this report is that a canine Leydig-cell tumor can: (a) produce estrogenic hormone, as does the canine Sertoli-cell tumor, (b) induce excessive feminization, and (c) give rise to metastases in the brain.

CLINICAL DATA

A male boxer dog, 7 years of age, was brought to us with the complaint that, instead of a left testis, it had a slightly protruding tumor in the left inguinal region. This tumor had been slowly developing during the preceding two years, and during that period the male dog had gradually shown symptoms of excessive feminization accompanied by impotence and complete loss of libido.

Feminization

The symptoms of feminization observed in the dog were as follows:

(a) *Nipples.* The twelve nipples, which previously had been hardly noticeable, increased in size to such an extent that the hind nipples attained a diameter of 5 cm. and the forenipples a diameter of 3 cm. (Fig. 1). During the last month they had been regularly secreting milk, and colostrum was readily produced on pressure.

(b) *Preputium.* The penile sheath had become pendulous (Fig. 2), and the owner reported that male dogs were perversely interested in his dog because of its preputial secretion and fought for the privilege of mounting it.

(c) *Female pattern of micturition.* The dog, which had previously, like every male dog, performed its micturition on three legs, had changed its habit gradually with progressing feminization. It started urinating by lifting its left hindleg, but finally decided to put it down on the floor and pass the urine in a typically female position (Fig. 3). During the

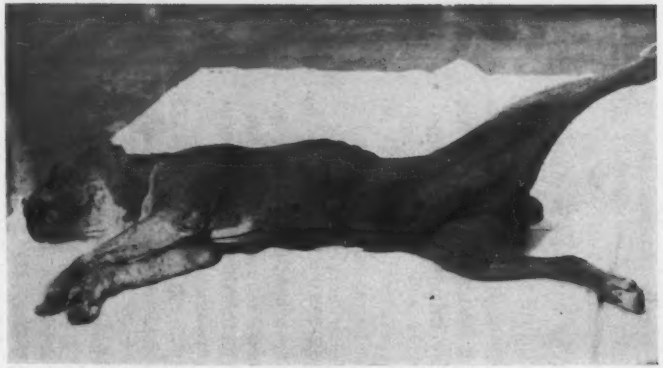

FIGURE 1

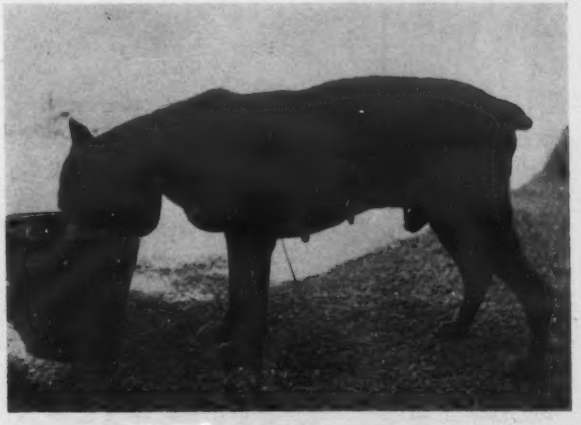

FIGURE 2

FIGURE 3

FIGURE 4

FIG. 1. Male dog (boxer) with twelve enlarged nipples.
FIG. 3. Micturition of the male dog in a typically female position.

FIG. 2. Pendulous penile sheath with constant discharge due to excessive estrogenization.
FIG. 4. Alopecia due to increased estrogen level.

last month the dog had finally entirely adopted the female pattern of micturition, *i.e.*, a position in between standing up and sitting down on his hindlegs.

(d) *Alopecia.* Marked eczematous inflammatory lesions of the skin developed from time to time although the animal had never suffered from eczema in earlier years. The skin lesions (Fig. 4) were peculiarly zonal and bilaterally symmetrical, involving both thighs and gradually spreading from there up to the neck. The skin of the depilated areas appeared smooth, pliable and somewhat thinner than normal. The remaining skin area was soft and fine, displaying the typical tenderness of the skin after excessive es-

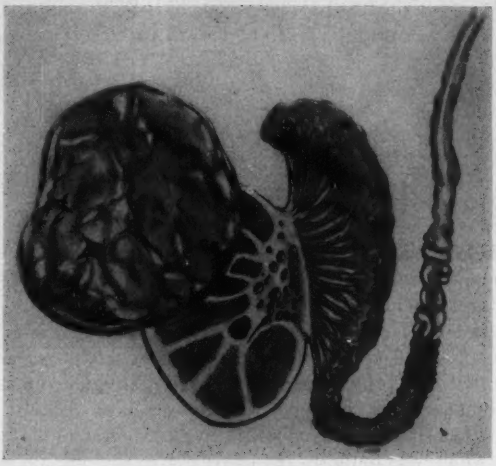

FIG. 5. The left testis is mostly destroyed and replaced by a large tumor mass (natural size).

trogenization. The cutaneous condition did not improve when a concentrated 14-day course of treatment was given, with 25-mg. of testosterone propionate and with 25 mg. of cortisone acetate intramuscularly per day.

OPERATION

An operation was performed in the left inguinal region and a small testis (2 × 3 cm.) with a large tumor (4 × 3 cm.) was removed (Fig. 5). The wound, under antibiotic treatment, healed *per primam.* Results of the histologic examination are listed in the section on postmortem examination.

The hormonal assays of the testis and of the tumor were performed after mincing the tissues, extracting with acetone in a Soxhlet extractor and dissolving the extract in olive oil. Three injections were given to castrated female mice, following the technique of the Allen-Doisy test. There was no evidence of estrogenic hormone in 2 grams of testicular tissue; the tumor, however, contained 1,000 I.U. of estrogenic hormone per gram.

FIG. 3. Micturition of the male dog in a typically female position.

FIG. 4. Alopecia due to increased estrogen level.
due to excessive estrogenization.

FOLLOW-UP

The condition of the dog was followed for two years after the operation. There was no change in the symptoms of feminization during the first year, but later these symptoms increased and became rather intractable. Assays of urinary estrogenic hormone yielded fluctuating values of 10,000–100,000 I.U. per day. Later on the animal showed a typical inclination for roaming, like a female dog in heat. When a peculiar type of ataxia with strange transient disorientation was added to these symptoms, indicating cerebellar metastases, it was decided to subject the animal to euthanasia by electrocution (10).

POSTMORTEM EXAMINATION

(a) *Gross examination*

The main findings proved to be in the remaining right testis, brain, liver and spleen.

1. *Testis.* The right testis measured 2×3 cm. in diameter; the tunica albuginea was smooth and glistening. On the cut surface the testicular structure could be detected in most areas. Multiple nodules, 0.5–1.0 cm. in diameter, were scattered in the tissue. These nodules were well delimited, yellowish in color and bulging on the cut surface with a homogeneous structure (Fig. 6).

2. *Brain.* The meninges were intact, except over the right lobe of the cerebellum, where they were thickened and adherent to a tumoral nodule measuring about 2 cm. in diameter (Fig. 7). The tumor nodule was friable, irregular on its external surface, and greyish and homogeneous on the cut surface.

3. *Liver.* The outer surface was smooth, except for one prominent mass of tissue, 5 cm. in diameter, which was found in the convexity of the organ. On the cut surface, a moderate congestion was noted. A cut through the described mass revealed a yellowish tissue with areas of hemorrhagic liquefaction, well delimited from the surrounding tissue (Fig. 8).

4. *Spleen.* On the diaphragmatic surface beneath the capsule there was a nodule measuring about 2 cm. in diameter, of a soft consistency (Fig. 9). On the cut surface this nodule was reddish in color. The cut surface of the rest of the spleen had no unusual appearance.

(b) *Histologic examination*

1. *Testis.* The seminiferous tubules showed poorly developed spermatogenesis and spermatozoa (Fig. 10). In between the tubules large areas of tumor nodules were present. The nodules consisted of epithelial cells of an alveolar pattern with a fine fibrillar stroma in between (Fig. 11). The cells were oval or round in shape, and large, with abundant eosinophilic cytoplasm and a round nucleus. The nucleus varied in size and was often eccentrically located. In many cells the cytoplasm was richly vacuolated, and contained sudanophilic granules in abundance. In the nucleus, nucleoli were scarce and no mitoses were detectable. In the stroma many capillaries were noted.

2. *Brain.* The histologic pattern of the tumor in the cerebellum was similar to that of the primary tumor nodule in the testis (Fig. 12).

3. *Liver.* The architecture of the liver in most areas was preserved and in some areas pseudolobules were present. The nodule described grossly turned out to be a large cavernoma.

FIGURE 6

FIGURE 7

FIGURE 8

FIGURE 9

FIG. 6. Right testis. On the cut surface are multiple tumor nodules.
Fig. 7. Cerebellum with a tumor metastasis in the right lobe.
FIG. 8. Liver with large cavernoma.
FIG. 9. Spleen, showing nodule of enlarged and engorged sub-capsular sinuses.

4. *Spleen.* Sections from different areas were examined. The structure of the spleen was preserved and a moderate congestion was noted. Sections through the single capsular nodule showed a proliferation of sinuses, noticeably congested, and large areas of recent hemorrhages.

(c) *Pathologic-anatomic diagnosis*

Interstitial (Leydig)-cell tumor of the left testis with secondary metastases in the right testis and cerebellum; gynecomastia and alopecia; large cavernoma in the liver; nodule of sinus proliferation, with congestion and recent hemorrhage in the spleen.

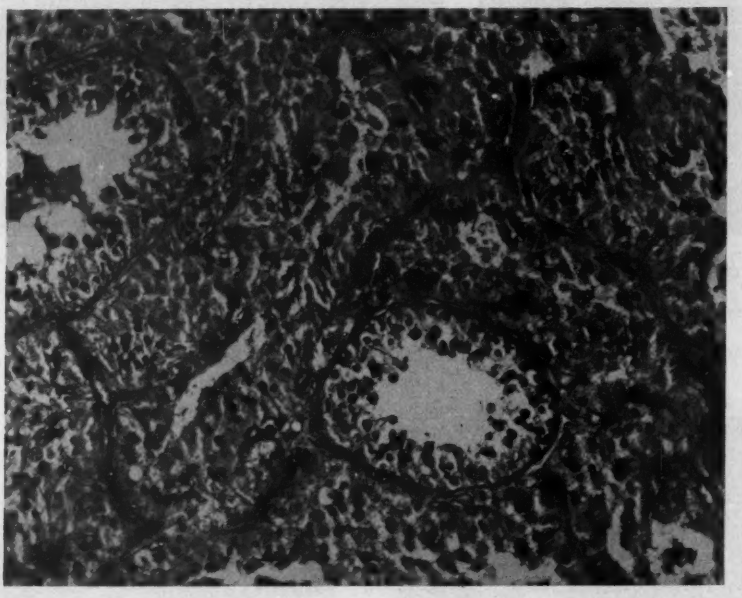

FIG. 10. Right testis. In the seminiferous tubules are seen moderate spermatogenesis and few spermatozoa between large areas of tumor cells. (×150.)

HISTOCHEMICAL STUDIES

The tumor and its brain metastasis were characterized as follows:

(a) Staining with Sudan III showed in the cytoplasm an abundance of sudanophilic granules (neutral fats).

(b) Examination under polarized light revealed no double-refractive bodies.

(c) Staining with the Ashbel-Seligman method (11, 12) yielded negative results.

These findings establish the presence in the cytoplasm of lipoid material without active carbonyl groups—probably estradiol.

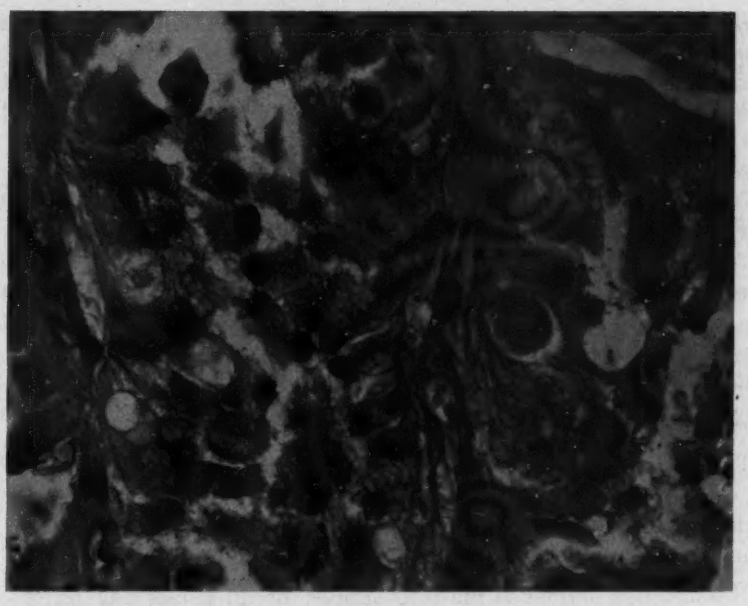

FIG. 11. Right testis. The tumor cells are large, nuclei are often eccentrically located and the cytoplasm is richly vacuolated. (×560.)

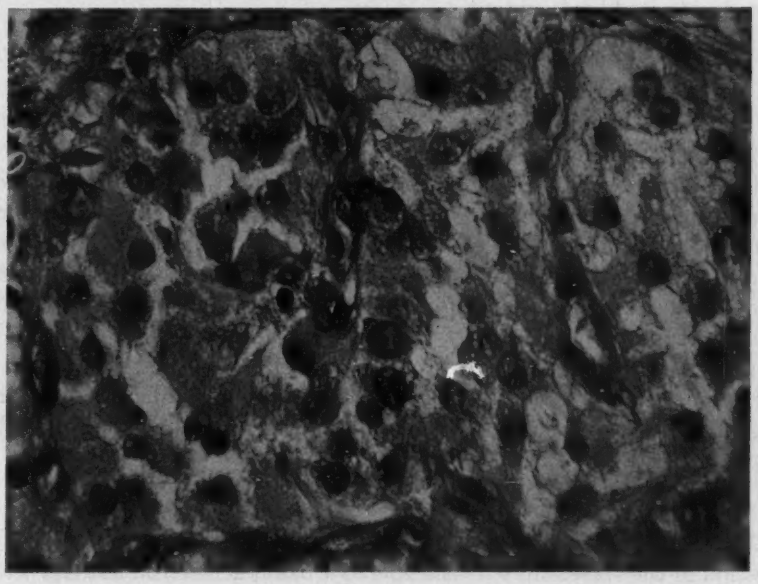

FIG. 12. Cerebellar metastasis. Note the similarity of the cells with those of the right testicular tumor. (×560.)

DETERMINATION OF HORMONES IN URINE AND IN LIVER HEMANGIOMA

Urine

On the day when the dog was sacrificed, a hormonal assay of the urine was carried out with the following results:

Follicle-stimulating hormone—less than 50 R.U./liter (normal).
Total estrogens (method of Sulman (13))—1,000 I.U./liter (increased).
17-Ketosteroids (method of Sulman (14))—6 mg./day (subnormal).
17-Hydroxysteroids (method of Sulman (15))—2.6 mg./day (normal).

Determination of estrogens (urine fractions) yielded the following values:

Estriol—55 gamma/liter (increased).
Estrone—53 gamma/liter (increased).
Estradiol—infinitesimal amounts.

Hemangioma of liver

The partly necrotic hemangioma of the liver was immediately assayed for estrogens in the following way: tissue weighing 17 grams was minced under acetone[1] and extracted with acetone in a Soxhlet apparatus; the acetone and water were evaporated and the remainder dissolved in 1.7 cc. of olive oil for the Allen-Doisy test. This tumor proved to contain only 1 I.U. of estrogenic hormone per gram of substance, which means that the tumor itself did not produce any hormone, its estrogenic hormone level being in fact due to its blood content.

DISCUSSION

1. *Metastases*

The most significant feature of the present Leydig-cell tumor was its tendency toward multiple metastases—in the second testis and in the cerebellum. The development of these metastases was accompanied by the reappearance of the symptoms of feminization associated with the primary tumor which affected the left testis removed by operation. In the case of Sertoli-cell carcinoma, the occurrence of metastases possessing the same endocrinologic function as the primary tumor has been demonstrated by Coffin, Munson and Scully (9). So far as we know, this is the first case in a dog in which metastases from a Leydig-cell carcinoma have been described and the first such tumor to possess full feminizing and endocrinologic functions. Only 6 metastasizing tumors of this type in man have been reported (16).

2. *Hemangiomata*

The huge hemangioma in the liver and the sinus proliferation with congestion in the spleen may be considered a banal accidental finding. This is,

[1] This precaution was necessary to avoid inactivation of the estrogens by liver estrinase.

however, rarely observed in the postmortem examinations of dogs. These findings have a certain similarity with the changes observed in the human body, in which excessive estrogenization following hepatic damage is always accompanied by mass appearance of arterial spiders (teleangiectasia) (17).

3. *Bisexual hormone production*

(a) *Estrogenic Sertoli-cell tumors.* Strong evidence exists that the male gonad can produce estrogen. Substances having estrogenic effect have been recovered from stallion, bull and human testes; and crystalline alpha-estradiol and estrone have been isolated from stallion urine and testes (18, 19). Whereas it is universally agreed that androgens are produced by the Leydig cells of the testis, the site of estrogen production has yet to be determined. Canine testicular tumors from which estrogenic hormone can be recovered in large quantities (8) are mostly tubular tumors of Sertoli-cell origin. They constitute strong evidence that these cells are capable of estrogen production in dogs (5). Teilum (20) reported a Sertoli-cell tumor in a human testis, similar morphologically to the canine tumor. It was associated with increased urinary estrogens and feminizing effects, such as gynecomastia, impotence, and loss of libido. Studies of other endocrine abnormalities of the human testis, and results of animal experimentation afford confirmatory data that the Sertoli cells are the source of an estrogen-like hormone (21). Experimental evidence for this fact has been brought forward by McCullagh and Schaffenburg (22); the substance they call "inhibin" seems to be estrogen. The histochemical studies of Ashbel and Seligman (11) and Ashbel, Cohen and Seligman (12) likewise furnish support for this conception.

(b) *Estrogenic Leydig-cell tumors.* There is no doubt that the tumor described by us is an estrogenic Leydig-cell tumor. Melicow and associates (23) in 1949 still stated that "interstitial cell hyperplasia and tumors are not uncommon in dogs, horses, mules, rabbits and in hybrid birds. They are prone to occur in cryptorchids and in testes of old animals. Endocrine disturbances, such as occur in humans, especially in children, were not described." In the meantime estrogenic Leydig-cell tumors have been found in animals. Kahan (24) recently described such a case in a dog. Human cases were described much earlier by Budd (25) and by Hunt and Budd (26). A review of the 10 human cases has been published by Melicow *et al.* (23).

These findings assume special importance through the studies of Maddock and Nelson (27). They showed that administration of chorionic gonadotropin (LH) to the human male causes an increase in urinary estrogen output, the histologic counterpart of which is a hyperplasia of the Leydig cells; the Sertoli cells do not show hyperplasia in such cases. Moreover, this increase in estrogen output can occur in an individual suffering from Addison's disease (suggesting that the adrenal cortex does not play an im-

portant rôle in the estrogen production), but does not occur in a castrate. These investigations show that Leydig cells produce estrogens only under conditions of extreme stimulation, furnished in the case of Maddock and Nelson by the injection of 5,000 I.U. of gonadotropin per day (a dosage of 500 I.U. per day was ineffective). Furthermore, urinary 17-ketosteroids were increased in these cases only twofold, whereas estrogens increased fivefold to sixteenfold. This is clear-cut evidence that there is no conversion of androgens into estrogens. Under normal conditions, a testis cannot be expected to produce estrogen in its Leydig cells, and apparently it is the Sertoli cells which produce the estrogenic hormone appearing in the semen. For example, Diczfalusy (28) found estrone, 17-beta-estradiol and estriol (mostly in the free form) in human semen. His paper contains the relevant literature on this topic.

(c) *Hormonal ambisexuality.* These considerations would not be complete if we omit mentioning the well known fact that the hormonal secretion of both the ovary and the testis can easily be changed from androgenic to estrogenic, and vice versa, by transplantation of the gonads to other sites of the body. Steinach inaugurated this research when he showed the occurrence of so-called "F-cells" in the testes. Hill (29, 30), too, demonstrated this for the ovary in the following ingenious manner: he grafted ovaries into the ears of castrated male mice and found that normal growth and secretion of the seminal vesicles and prostate occurred. Abdominal ovarian grafts did not, however, produce this effect. As the temperature of the ear is lower by 5–6° C. than that of the abdomen, it seems possible that temperature may be a controlling factor in the androgenic activity of the ovary. Similar results were obtained by Hernandez (31), using a strain of female rats in which prostatic lobes never occur. Ovarian grafts on the tails of such animals induced marked growth of the prostate and clitoris. This investigator also obtained evidence that ovarian androgens are secreted by the theca cells.

It is, therefore, apparent that determination of the origin and nature of hormonally active tumors cannot always be based solely on their clinical pictures, but that the cytologic features of such tumors should be investigated and interpreted independently of the endocrinologic aspects of the case.

SUMMARY

A Leydig-cell tumor in a male dog is described, which presented the following peculiarities:

1. Development from a cryptorchid testis.
2. Formation of multiple metastases in the second testis and the cerebellum.

3. Presence of large hemangiomata in the liver and spleen.
4. Production of estrogenic hormone in amounts of 1,000 I.U. per gram of tumor tissue, and excretion of estrogen in the urine in amounts fluctuating between 10,000–100,000 I.U. per day. Excretion of 17-ketosteroids was decreased.
5. Extreme external feminization documented by marked enlargement of the nipples, a pendulous preputium, female pattern of micturition, alopecia, and complete loss of libido.
6. In the later stage of the condition this male dog showed a strange urge for roaming, resembling that of a female dog in heat.

The hormonal activities of both testicular and ovarian tumors are discussed. In view of the feminizing potentialities of testicular cells, plus the evidence that ovarian cells may be capable of producing androgens, tumors of the testis and the ovary should be classified by their histologic origin as well as by the type of hormone produced.

Acknowledgment

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SEX DIFFERENTIATION BY LEUKOCYTE MORPHOLOGY

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FOLLOWING the observations of Barr and Bertram in 1949 (1) on the neurones of cats, several workers have shown that the sex of certain mammals can be clearly identified from the microscopic appearance of the cells of various tissues (2–7). Graham (8) showed that in the embryo this specificity antedates sexual differentiation of the gonads. Moore and Barr (9) investigated numerous human tissues which demonstrate sex differences of morphology. Studies of the cell nuclei of the skin in biopsy specimens have been used in investigations on hermaphrodites and other ababnormal individuals (10–13). The sex morphology of teratomas and other tumors is under study (14–17) and preliminary reports have appeared on the sex differences of the cells in scrapings of the oral mucosa (18, 19).

Davidson and Smith (20) showed that there is a morphologic sex difference in neutrophil leukocytes of normal individuals of widely different ages. They did not claim, however, that leukocyte morphology was necessarily an indication of chromosomal sex. The present study was undertaken to confirm and extend their findings and to study the relationship of leukocyte morphology to various endocrinopathies, dysgenetic states and congenital anomalies. It was hoped that these findings might lead to the development of an additional diagnostic tool and provide a method of sex differentiation for sexually indeterminate individuals, especially in the newborn. Such a method would prove to be a more useful clinical test than skin biopsy, since blood lends itself to simple examination, is readily available and may be obtained as often as necessary with little or no discomfort to the patient.

BASIC DATA

Davidson and Smith noted that in the female the nucleus of the mature neutrophil leukocyte possesses more frequent chromatin condensations than are seen in the male. One such condensation, the "drumstick," is a dis-

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tinct appendage entirely distinguishable from the rest of the nucleus, to which it is attached by a fine stem (Figure 1). The head is rounded, stains darkly and measures approximately 1.5 μ in diameter. Typical drumsticks are never found more than once in the same cell and, on the average, are present once in every 38 mature neutrophil polymorphs. They are to be

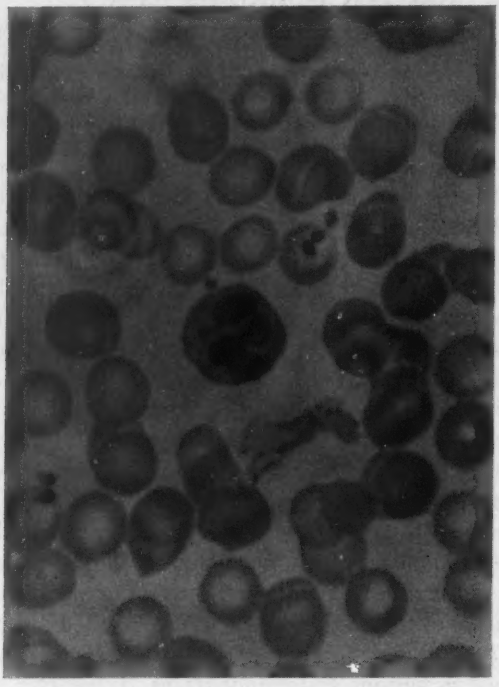

FIG. 1. Typical drumstick projecting from the smaller of the two nuclear lobes.

distinguished from "small clubs" (less than 1 μ in diameter) (Figure 2) and from accessory lobes of the nucleus (Figure 3). Davidson and Smith found at least 6 drumsticks during the examination of 500 neutrophils in the female and were unable to find a single drumstick in 500 neutrophils in the male. Two of their cases gave them difficulty. Their observations of 500 patients suggested that the incidence of drumsticks increased with greater segmentation of the leukocytes. Conversely, when nuclear segmentation was diminished, as shown by a marked shift to the left (not defined), there was a reduction in the number of drumsticks. In such an instance, it would be possible to examine 500 cells in the female without finding as many as 6 drumsticks. Thus, the findings take on qualitative and quantitative as-

pects. In our study, attention was directed chiefly to drumsticks, but the other condensations of chromatin, particularly those in the form of "sessile nodules" (probably characteristic of the female), were also observed.

METHOD OF STUDY

One investigator first familiarized himself with the method of sex differentiation to be used in the study and made a preliminary verification of its accuracy by correct

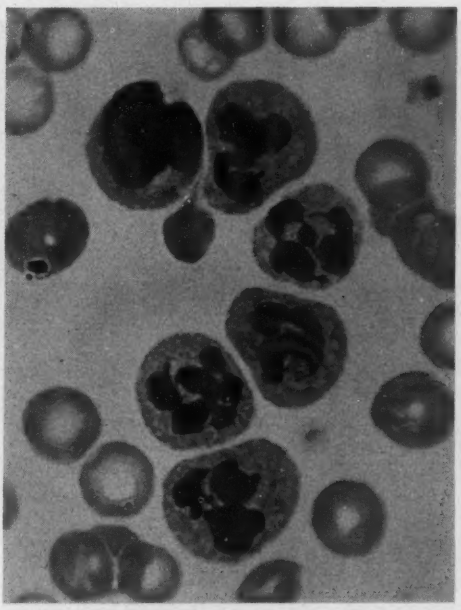

FIG. 2. Numerous projections are seen on the nuclei of these cells. Not one of them is a true drumstick; they are all "small clubs."

sex diagnosis involving 40 unknown slides which have not been included in this series. He then systematically examined and reported on the slides of the present series which were made from 46 normal persons and 87 patients attending the endocrine clinic. All the slides were arbitrarily numbered to conceal identity. In a great many instances, blood from the same patient was submitted under two or more different numbers. In every case, 500 neutrophils were examined with the oil immersion lens, unless 6 drumsticks had been identified before this number was reached. A carefully worded report was composed for each slide, concluded by a diagnosis of "Male" or "Female." Where less than 6 drumsticks had been seen in 500 neutrophils, "No conclusion" was reported. Only after the final conclusions had been drawn did the examiner receive any clinical information about the patients.

In order to evaluate the importance of shifts to the left[1] or right in the number of

[1] Shift to the left—a decrease in nuclear segmentation.

nuclear lobes, a large number of lobe counts were made under equally objective conditions.

The cases studied are listed in Table 1. The findings are summarized in three columns: (a) when the clinical sex was in agreement with the conclusion drawn from examination of the slide according to the strict criterion of at least 6 drumsticks per 500 neutrophils, (b) when drumsticks were found but the incidence was less than 12 per 1,000 neutrophils, and (c) when the sex predicted from the slide was the opposite of the clinical sex.

Throughout the series it was imperative to insist upon good technique, having regard

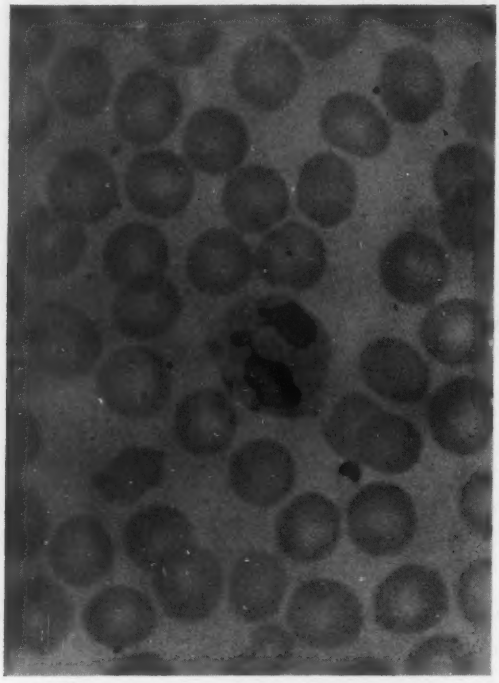

FIG. 3. The projection borne on a thin stem is an accessory lobe of the nucleus, not a drumstick.

for the difficulties inherent in making and interpreting any blood smear (21, 22) and for the distortion which polymorphs readily undergo with faulty handling or during drying (23). The quality of the smear necessary for this work is much higher than is required for the usual differential count.

RESULTS

Normal males (17 cases)

In none of the normal male subjects was any drumstick found. There was also a complete absence of sessile nodules and in every instance the correct diagnosis of male sex was made from the blood smear.

TABLE 1. ANALYSIS OF 133 CASES STUDIED

	(a) Conforming exactly to criteria	(b) Not conforming exactly, but diagnosable*	(c) Showing pattern of opposite clinical sex
Normal male (19 cases)	19		
Abnormal male (22 cases)			
Gynecomastia (various types)	5		
Klinefelter's syndrome	4		
Ectodermal dysplasia	1		
Hypogenitalism	2		
Eunuchoidism	3		1
Mongolism	2		
Gargoylism (Hurler's syndrome)	1	1	
Addison's disease	1		
Orchidectomy (massive estrogen) therapy)	1		
Normal female (27 cases)	27		
Abnormal female (62 cases)			
Adrenogenital syndrome	6		
? Adrenogenital syndrome	1	1	
Stein-Leventhal syndrome	3		
Chiari-Frommel syndrome	2	1	
Albright's syndrome	2		
Cushing's syndrome		1	
Cushing-like syndrome	2		
Primary amenorrhea	5		
Secondary amenorrhea	7	3	
Obesity with oligomenorrhea	1	1	
Oligomenorrhea		2	
Hirsutism	1		
Hypopituitarism		1	
Hypothyroidism	1	1	
Mongolism		1	
Undiagnosed		1	
Gonadal dysgenesis (ovarian agenesis)	10	1	7
Unknown sex (3 cases)			

* In the cases included in column b, drumsticks were seen on the polymorphs, but with a frequency of less than 6 per 500.

Abnormal males (22 cases)

Examination of all but 2 of the abnormal males listed in Table 1 yielded equally uniform results. There was an entire absence of any nuclear features which would have suggested the female pattern. Several of these individuals showed many distinctly feminine physical characteristics. Of the 5 cases of gynecomastia, 3 showed marked glandular hyperplasia and hypertrophy. The findings in the 3 patients with Klinefelter's syndrome, all of whom had complete azoospermia associated with increased excretion of gonadotropic hormone, are interesting when compared with the findings in gonadal dysgenesis, since there is failure of gametogenesis in both instances.

In the patient with ectodermal dysplasia, there was complete absence of body hair, eyebrows and eyelashes, sparse cephalic hair, depigmentation of the skin and retinae, photophobia and a total of four teeth. Neither the patient with hypogenitalism, who had previously been treated with large doses of chorionic gonadotropin, nor the man who had undergone orchidectomy and was receiving large doses of estrogenic hormones, showed any exception to the male pattern.

One case, however, gave some difficulty; the neutrophils of a 4-year-old boy with gargoylism showed very occasional projections similar in appearance to drumsticks, though not entirely characteristic. In one blood smear from this boy, 500 neutrophils were examined without any of these appendages being seen, and a report of "male" was made. In another of his blood smears two such appendages were seen and reported with a statement that no conclusion could be reached.

In 1 male patient the results were unique. The clinical diagnosis was eunuchoidism and he had the typical external appearance associated with that syndrome. The penis was small, the voice high-pitched and the escutcheon was of the female type. Atrophic appendages were present in the scrotum. The patient had never had genital surgery nor had his scrotal contents been examined prior to a recent biopsy. He was married but had no children. The blood smear showed the female pattern with 6 drumsticks in 205 neutrophils (confirmed on two occasions) and did not appear different from the smears of normal women. Biopsy of the scrotal contents showed an indeterminate histologic picture. Urinary excretion of follicle-stimulating hormone was greater than 105.6 mouse units per twenty-four hours.

Normal females (26 cases)

In the blood smears of all the normal females, including women of Negro and Oriental ancestry, 6 drumsticks were seen before 500 neutrophils had been examined. In addition, numerous sessile nodules and other condensations and projections of chromatin were noted; their distribution is being

studied further. Although the frequency of drumsticks was within the limits given by Davidson and Smith, one point emerged from our work which is not clear from their report. From our figures it appears that the frequency of drumsticks is a characteristic of the individual; thus, in some subjects examined repeatedly, it would never be necessary to count more than 150 neutrophils to find 6 drumsticks, but in others it would always be necessary to count, say, 350 to 500 neutrophils.

Abnormal females (62 cases)

All the patients living as women had blood smears recognizable as female, although in many of them the number of drumsticks was lower than the critical 6 per 500 neutrophils which had been chosen on the basis of Davidson's report. There were 7 exceptions—all cases of gonadal dysgenesis (ovarian agenesis) which had the male pattern.

Cases conforming exactly to criteria

The various abnormal states listed in column (a) of Table 1 were not associated with departures from Davidson's criteria. Included among these were 5 patients with marked masculine characteristics typical of the classic adrenogenital syndrome associated with adrenocortical hyperplasia. In 1 of them the clitoris was of penile dimensions. Most of these patients were receiving cortisone at the time the blood smear was made. Of the 2 subjects in whom the diagnosis of adrenogenital syndrome was more doubtful, 1 showed a reduced number of drumsticks in the blood smear.

There were 3 women with the Stein-Leventhal syndrome, in all of whom there was gross and microscopic evidence of the severe ovarian disturbance which appears to be the central defect of the syndrome. The blood smears were indistinguishable from those of other women.

Low incidence of drumsticks

The patients listed in column (b) of Table 1 formed an interesting group. During the examination of each smear, 500 neutrophils were counted without the finding of 6 drumsticks. The reports carried no final conclusion regarding sex, but in retrospect it may be seen that each case was diagnosable as female, not only because there were at least some drumsticks, but also because of the frequency of sessile nodules and other female features. In doubtful cases the first examination of 500 cells raised enough suspicion to prompt a further investigation of several hundred more. From these supplementary examinations, the average number of drumsticks per 1,000 neutrophils was calculated. One case of Cushing's syndrome gave rise to difficulty. Numerous and repeated counts for this patient showed drumsticks in a frequency of 2 per 1,000 neutrophils. It is not surprising, therefore, that on one

occasion 500 neutrophils were examined without the finding of a single drumstick and the conclusion was drawn that the patient was male, on the basis of a strict application of the original criteria. However, the frequency of sessile nodules was such that the experienced observer would have wanted to examine more than 500 cells before reaching a conclusion. The same difficulty occurred in 1 case of gonadal dysgenesis.

Some of the patients whose blood smear showed few drumsticks also showed a shift to the left in the lobe count, but this was by no means consistent. The significance of a low drumstick count and its correlation with the lobe count are discussed later. The cases in this category make up a heterologous group of women with no definite abnormal features or endocrinopathies in common. One patient had a premature climacteric syndrome associated with an increased excretion of follicle-stimulating hormone and premature ovarian failure. She had menstruated normally until the age of 23 when, following mental trauma associated with the death of her fiancé, the menses ceased completely, though they were readily re-induced by steroid hormone replacement therapy. Another patient was short and obese and had an accentuated fat pad in the cervical area but no purplish striae. The arms and legs were thin in comparison with the rest of the body and she had mild hypertension and oligomenorrhea. At the time of examination she had a 4-year-old child, but was experiencing difficulty in conceiving a second time. Since then she has become pregnant. The possibility of a Cushing-type syndrome was considered, but diagnosis must await further data. Other women in this group suffered from obesity, hirsutism and menstrual disorders. If they had anything in common, it is the strong suggestion of an endocrine disturbance.

Gonadal dysgenesis (ovarian agenesis)

Although it may be argued that the diagnosis of gonadal dysgenesis may be proved only at laparotomy or autopsy, in the 18 cases studied a convincing diagnosis was based on several criteria. The patients were usually of short stature and had primary amenorrhea. Urinary excretion of gonadotropic hormone was increased in the majority, but in others it was normal or reduced. In 6 cases there was webbing of the neck. In many, the absence of functioning ovaries was established by the use of pregnant mare's serum or by culdoscopic examination. The clinical facts are summarized in Table 2.

In 7 cases the blood smear showed the typical male pattern. Blood from these patients was examined again and again under different serial numbers and at no time was there any report of drumsticks or sessile nodules of chromatin.

In 10 cases the pattern was emphatically female and it was remarkable

TABLE 2. ANALYSIS OF 18 CASES OF GONADAL DYSGENESIS

Subject No.	Age (yrs.)	Height (ins.)	Weight (lbs.)	Urinary gonadotropins (m.u.)	Urinary 17-ketosteroids (mg./24 hrs.)	Response to pregnant mare serum	Menstruation	Webbing of neck	Hormones in use when blood smears taken	Remarks	Blood smear	
											Sex	Frequency of drumsticks in neutrophils
111	23	58	53	105.6	6.4	None	Never	Yes	Estrogen progesterone		M	—
113	17	61	126	under 105.6	16.3	None	Never	—	None		M	
67	18	54	90	over 211	4.9	—	Never	Yes	Estrogen, progesterone		M	—
19	28	58	104	over 211	4.5	—	Never	—	Estrogen, progesterone		M	—
30	17	50	87	over 211	2.1	None	Never	Yes	Estrogen, progesterone		M	—
171	20	57	128	—	8.6	None	Never	Yes	Estrogen, progesterone	Negro	M	—
179	36	60	126	—	5.9	—	Never	—	Estrogen, progesterone		M	—
95	19	56	79	—	—	—	Only during therapy	Yes	Estrogen, progesterone		F	6/248
106	25	65½	205½	over 105	4.2	None	Never	—	None		F	6/48
189	36	63	133	52.8	6.9	None	Never		Progesterone	Edema	F	6/184
73	24	60	155	under 6.6	4.5	None	Never		Estrogen, progesterone	Edema	F	6/97
180	48	62	139	under 26.4	5.1	—	Never		None		F	6/63
102	25	66	151	—		None	Never	—	Estrogen, progesterone		F	6/96
83	33	62	140	over 105	8.9	None	Only during therapy	—	Estrogen, progesterone		F	6/116
40	32	58	115	over 211	6.0	None	Never	Yes	Estrogen, progesterone		F	6/160
45	19		135	52.8	9.7	None	Only during therapy	—	Estrogen, progesterone		F	6/160
64	32	64	120	over 105	23.	None	Never	—	Estrogen, progesterone		F	6/32
86	21	65	142	under 6.6	6.1	None	Only during therapy	—	None		(F)	4/1,000

that in 8 of these the frequency of drumsticks was unusually high, so that it was possible to find 6 drumsticks in counting no more than 160 neutrophils at most. One patient showed 1 drumstick for every 5 neutrophils. The patient with gonadal dysgenesis who showed only 8 drumsticks per 1,000 neutrophils has been mentioned in a previous paragraph.

Thus, the cases of gonadal dysgenesis fell very sharply into two categories, male or female, on the basis of polymorph morphology. Surveying the patients in retrospect it is impossible to assign them to these two groups in terms of any other clinical or laboratory data. All 18 of them responded well to cyclic estrogen and progesterone therapy, with the development of female-type maturation, fat distribution and hypertrophy of the breasts. It is noteworthy, however, that in 6 of the 7 patients showing the male pattern, in whom the excretion of gonadotropin was studied, the values were distinctly elevated. The output of 17-ketosteroids was variable from case to case.

The highest drumstick count recorded in this study (6 in 21 neutrophils) was in a woman with amenorrhea and the neck-webbing typical of Turner's syndrome, who could not be said to have true gonadal dysgenesis since spontaneous menstruation had occurred in the past.

Infants of unknown sex

Three infants were referred for examination because their external appearance left the sex in doubt. Repeated examination of the blood from one of them showed no drumsticks or sessile nodules. The second showed 6 drumsticks in 61 neutrophils, and the third showed 6 in 245. The interpretation of these findings is discussed later.

DISCUSSION

The results in this series show a striking consistency, which speaks for the validity of the original premises. The data are accurate and categorical in their confirmation of Davidson's original findings in normal individuals. This is truly remarkable when it is remembered that the normal leukocyte count is subject to wide variations (24–26). Fluctuations in number and appearance of the cells have been familiar to hematologists for a long time, but so manifold are the changes that they have defied many efforts at accurate classification and description. The Arneth count and its modifications, which enumerate the nuclear lobes, yield widely discrepant results and provide, at best, an inexact picture of the prevailing type of nucleus in the polymorphs of a smear. Since the classification of nuclei according to the number of lobes is largely a matter of personal judgment, reports of normal ranges of values are few, and these are at variance with one another.

Furthermore, one patient's polymorphs may show a complicated pattern of nuclear chromatin and another's may have smoother, simple nuclear lobes; yet the lobe count may be the same for both. Clearly, there is much in the detail of nuclear structure which remains to be studied.

The various appendages to the nuclei have received little attention since the writings of Knoll (27), but interest in them has been renewed since Davidson and Smith showed the presence of drumsticks to be related to the patient's sex, as also are sessile nodules and perhaps certain other features of the nucleus. The significance of small clubs and other appendages remains unknown.

The factors determining how many of the neutrophil nuclei bear drumsticks are obscure, but observations in the present series suggest that the frequency is a function of the individual. The distribution of drumsticks was found to be remarkably constant when patients were repeatedly reexamined at three-month intervals or less. The findings are interesting in view of the evidence given by Turpin *et al.* (28–30) that the lobe count is a function of the individual. Furthermore, it is conceivable that the lobe count is genetically determined, since the observations of Undritz (31–35) on families showing nuclear hypersegmentation, and similar studies in rabbits afford much evidence of hereditary control of nuclear segmentation.

A special reason why knowledge of the frequency of nuclear drumsticks becomes important is the desirability of establishing an infallible test for nuclear sex. In his series, Davidson always found 6 drumsticks before 500 neutrophils had been examined, though 2 cases gave rise to difficulty. The present study supplies similar findings, but the number of normal subjects is so small that it is unjustifiable to speculate on how often normal individuals fulfill the criteria. As far as abnormal individuals are concerned, the criteria are definitely not always fulfilled, so the question arises as to how many neutrophils must be scrutinized before a definite sex diagnosis can be made. In retrospect, we may rewrite our criteria to say that if 500 neutrophils have been counted and no drumsticks have been seen, then the chromatin pattern is male unless (a) there is a marked shift to the left, or (b) sessile nodules or other chromatin condensations of the type seen in the female have been noted. If either of these conditions is present, the examination should proceed to 1,500 cells, and when drumsticks are found the chromatin pattern may be said to be female. If these criteria had been adopted, a nuclear sex corresponding to the clinical sex would have been assigned to every individual in this series, with the exception of 7 cases of gonadal dysgenesis and 1 case of male eunuchoidism; the single difficulty would have been the boy with gargoylism (who is discussed subsequently). It may be postulated that the finding of one drumstick alone is enough to

establish the diagnosis "female" and, as far as this survey is concerned, that would be true. In general, however, no hematologic diagnosis is secure when it is based on the findings in a single cell and any conclusion drawn from a blood smear rests ultimately upon the observer's judgment. It is best that the examination proceed, whenever possible, to the finding of 6 drumsticks.

The syndrome of gonadal dysgenesis (ovarian agenesis) has been discussed in numerous recent articles (36–37). The observed facts are consistent with the explanation of Grumbach *et al.* (38)—that gonadal failure occurs in early fetal life, after which development proceeds according to an inherent intra-uterine tendency to femininity, just as castrated embryonic mice and rabbits develop as females. If this is so, then in the absence of any suggestion that one sex is more susceptible than the other to gonadal failure, one would anticipate that the genetic sex distribution in gonadal dysgenesis would be 1 male to 1 female. Using the skin biopsy technique, Wilkins *et al.* (39) reported 20 cases as male and 2 as female. Polani and Magnus (40, 41) mention 9 cases showing the male pattern, but do not state whether any of their cases showed the female pattern. Rannie and Erskine (42) mention 1 case showing the female pattern, and Russell *et al.* (43) 1 that showed the male configuration. Barr (44) found the male skin pattern in 16 patients and the female in 5. In our series the blood chromatin pattern was female in 11 cases and male in 7 cases. A greater accumulation of results will be necessary before the distribution is finally settled but, so far, neither skin biopsy nor the blood examination has yielded results which can be relied on as an unmistakable indicator of genetic sex. It is difficult to give a ready explanation for the finding of a "female" blood smear in the 1 case of eunuchoidism. Damage to the gonad may have been due to childhood surgery but it is possible that failure of the gonad had occurred in utero and thereafter development had proceeded along male lines rather than along the female lines seen in gonadal dysgenesis (note the high excretion of FSH). The mother was dead when the patient was seen but there was no evidence of her having had any virilizing condition or virus infection during pregnancy, or of there having been a male twin which might have exerted a developmental influence such as in the case of the freemartin in cattle.

Further studies are necessary in order to relate the findings in blood smears to those in skin biopsy specimens, and to see how far they agree. If some change occurs during embryonic life, then its timing or mode of operation may be such as to effect leukopoiesis but not to influence an already established skin pattern. Examinations of tissues by the methods described by Graham (8), particularly with regard to the microscopic anatomy of the uterus in cases of gonadal dysgenesis, will further elucidate the problem.

Relationship of leukocyte morphology to dysgenetic states

Since many abnormal subjects in this series showed drumstick counts of an individual character, it may be asked whether the nuclear appearance of the polymorphs may contribute to the diagnosis of any particular endocrinopathy or dysgenetic state. In this context, three examples of the association of leukocytic anomalies with physical deformities should be borne in mind: i) It has been shown (29, 30, 45) that in mongolism there is a shift to the left in the lobe count, though many normal individuals show shifts to the left of a similar degree. ii) The Pelger-Huët anomaly of reduced segmentation of the polymorphs is due to an autosomal dominant gene which is present in the heterozygous state. Nachtsheim (46) studied the syndrome in rabbits and found that the homozygous rabbit usually dies in utero or else is born in a state of gross physical deformity with shortening of the limbs and bony abnormalities. Begemann and Campagne (47) reported a marriage between 2 human heterozygous carriers of the anomaly; 2 miscarriages occurred and 3 children died in early life, before a homozygous daughter was born who was physically normal, apart from epilepsy. iii) It was shown by Reilly (48) that an anomaly of the granulation of the leukocytes of a type later described by Alder (49) and others (50, 51) was often associated with gargoylism. Undritz (32), in a review of the subject, found that the Alder anomaly of the leukocytes is often familial and that about half of the reported cases are associated with gargoylism.

From the present survey, three pertinent observations may be selected: 1) In the cases of gonadal dysgenesis showing the female pattern, the occurrence of drumsticks was very frequent. In Table 3 the frequency of drumsticks in this group is compared with that in other groups. In a case of secondary amenorrhea with webbing of the neck, 6 drumsticks were found in 21 neutrophils. 2) The only male patient causing any difficulty was a case of gargoylism. Immediately following examination of the patient's

TABLE 3. FREQUENCY OF DRUMSTICKS IN DIFFERENT CLINICAL GROUPS*

Diagnosis	No. of cases	Mean frequency of drumsticks
Normal female (Davidson and Smith)	225	1:38
Normal female (Present series)	14	1:43.6±15.1
Gonadal dysgenesis (with typical female pattern)	10	1:20.6±11.25
Adrenogenital syndrome	6	1:35 ±15.8
Stein-Leventhal syndrome	3	1:34 ±19.4

* Only those cases were used in which the count of drumsticks was known to be accurate, from the use of perfect smears and from repeated checking of the same patient.

blood, an examination was made of the blood from a normal sister. The investigator was impressed by the similarity of the nuclear chromatin in the 2 cases, despite his ignorance of the origin of the smears. 3) When the distribution of drumsticks was unusually sparse, there was a suggestion of some endocrine disturbance, regardless of whether or not there was a shift to the left in the lobe count.

These observations will direct our future studies to the exploration of the following possibilities: 1) A high drumstick count in the leukocytes of an individual living as a woman may suggest the diagnosis of gonadal dysgenesis, though possibly no more so than a shift to the left in the lobe count may suggest mongolism. 2) Further studies of gargoylism may reveal specific nuclear anomalies in patients or families, similar to the cytoplasmic granulation anomalies known to be associated with the syndrome. 3) Unusually low drumstick counts may be related to pituitary or other endocrine dysfunction. This would indicate either an influence of the endocrine system on the number of drumsticks or else that some forms of endocrinopathy (*e.g.*, mongolism) are dysgenetic states associated with anomalies of the nuclear morphology of the leukocytes. Studies of familial gonadal dysgenesis and other syndromes might prove helpful. However, Luers (52) has shown that the abnormally low nuclear segmentation of polymorphs occurring in mongolism is not a familial trait.

Subjects of unknown sex

Extreme caution should be exercised in the application of the data from blood smears to the 3 infants of unknown sex. The absence of drumsticks in the blood from 1 child and their presence in the blood from the other 2 suggested, at first sight, 1 boy and 2 girls. In fact, however, any of them may subsequently present the features of gonadal dysgenesis on the one hand, or eunuchoidism on the other. Indeed the possibility of gonadal dysgenesis is strengthened in 1 of them by the finding of 6 drumsticks in 61 neutrophils. Since the infants were known to be abnormal from their external appearance, they may be described as dysgenetic individuals and it is precisely in these circumstances that great caution is to be used in assigning a sex (53). Armstrong (54), in his review of the subject, emphasizes that there is no definition of sex and that some patients cannot easily be designated male or female. All of our patients living according to a sex opposite to that indicated by their nuclear chromatin were happier and better adjusted to life than they would have been if they had been reared as members of the other sex. In both groups of patients with gonadal dysgenesis it has usually been possible to induce marked femininity by the use of ovarian hormones. Nothing would be more deplorable than to allow the findings of the skin or polymorph examination to become the sole or over-

riding arbiter of an individual's sex. However, it is hoped that these tests will provide evidence which may be weighed with other conflicting information in solving the difficult clinical problems of intersex.

SUMMARY

1. Davidson and Smith's finding of sex differences in the nuclei of polymorphonuclear leukocytes has been used in a study of endocrinopathies and dysgenetic states.

2. In 46 normal control subjects, as well as in numerous others not reported here, the findings as originally stated are entirely confirmed.

3. In 87 abnormal patients, sex was diagnosable from the blood smear in all but 8 cases, in each of which the blood smear indicated the opposite of the sex by which the patient lived. In 15 subjects, however, it was necessary to modify the criteria.

4. The incidence of "drumsticks" on the nuclei of neutrophils appears to be related to the physically abnormal conditions involving endocrine disorders, and should be further investigated.

5. Gonadal dysgenesis (ovarian agenesis) is divisible into "male" and "female" types, according to the nuclear chromatin pattern. Clinically, however, the two groups of patients are indistinguishable.

6. A patient with eunuchoidism living as a male showed the female leukocytic chromatin pattern.

7. Findings in the blood smears from 3 infants of unknown sex are discussed.

8. Some possibilities for future investigation are outlined.

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FURTHER OBSERVATIONS ON THE ESTIMATION OF URINARY GONADOTROPINS IN NON-PREGNANT HUMAN SUBJECTS

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ONE of the great difficulties in estimating the excretion of gonadotropins in non-pregnant subjects is the fact that no International Standard preparation is available for comparative assay of these substances. Accordingly it has been necessary to express results of clinical assays in terms of arbitrary "animal" units, a unit being defined as that quantity necessary to produce a given effect. Arbitrary units employed in intact and hypophysectomized rats have included the doses necessary to induce vaginal cornification, to cause histologic changes in the ovaries, and and to induce a 50–100 per cent increase in the weight of ovaries, uterus, seminal vesicles and the ventral lobe of the prostate (1–4). In intact immature mice most investigators have adopted as a unit the quantity of extract causing a 100–200 per cent increase in uterine weight (5–8). It is now generally recognized that the error of results expressed without reference to a standard preparation may be threefold or more, and that little quantitative significance can be attached to data calculated in this way.

The excretion of gonadotropins is low in men and in women during reproductive life. Excretion is generally high, however, in women at any age beyond the menopause and it is therefore relatively easy to prepare potent gonadotropic extracts from the urine of these subjects. Part of the present investigation was undertaken to ascertain whether it would be a valid procedure, as far as statistical criteria are concerned, to use a gonadotropin preparation from menopausal and post-menopausal urine (Human Menopausal Gonadotropin—HMG)[1] as a standard for the comparative assay of gonadotropins from males and from non-pregnant females. Preliminary studies had indicated that the existing standard preparations for Human Chorionic Gonadotropin (HCG) and for Pregnant Mare's Serum Gonadotropin (PMSG) could not be used for this purpose.

A further difficulty in estimating gonadotropins in the urine of men and of women during reproductive life is that, owing to the very small amounts

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[1] This term will be used to designate the gonadotropic activity in the urine of menopausal and post-menopausal women. Such urine extracts contain both FSH and ICSH activity. The term HMG was introduced at the first meeting of the "Gonadotrophin Club" in Geneva, Switzerland (August, 1953).

of activity present, it is necessary to prepare concentrated extracts which are often toxic to the test animals. The various methods proposed for reducing toxicity, *e.g.*, by dialysis, have usually resulted in a loss of activity (6). Therefore the investigation has also been concerned with quantitative methods of extraction of the gonadotropins from urine so that the final product for bioassay is sufficiently active and, at the same time, relatively non-toxic. An investigation of the behavior of HMG in the kaolin-acetone extraction method, and in the tricalcium phosphate chromatography procedure of Butt and Crooke (9), was reported in an earlier paper (10). It was shown that for HMG, accurate control of the pH is essential at both the adsorption and elution stages of the kaolin procedure and that treatment of crude extracts of HMG with tricalcium phosphate was a useful means of removing inactive and toxic materials. The study has now been extended to include the gonadotropins derived from the urine of normal men, and of women during the follicular and luteal phases of the normal menstrual cycle.

Preliminary accounts of this work have already been given (11, 12). A quantitative method for estimating the gonadotropin content of the urine of human non-pregnant subjects, based on these findings, will be described in detail in a later publication.

MATERIALS AND METHODS

A. Biologic

(i) *Standard preparation—HMG-20*

This was prepared by Messrs. Organon (Newhouse, Scotland) from the urine of menopausal and post-menopausal women. Pooled urine was extracted by the kaolin method with subsequent fractional precipitation by acetone. The crude kaolin-acetone extract was treated with tricalcium phosphate according to the method described by Butt and Crooke (9), in order to reduce toxicity. The final powder was freely soluble in water; it was relatively non-toxic and could be administered to intact mice and hypophysectomized rats up to a total dose of 16.0 mg.

(ii) *Methods of assay*

Parallel assays on the same urine extracts were conducted by:

(a) The test depending on the *enlargement of the uterus* in intact immature mice (5, 8). This method, although sensitive, is not specific for either FSH or ICSH activity.

(b) The method depending on the *enlargement of the prostate* (ventral lobe or total gland) in hypophysectomized immature rats (4, 13). This method is generally assumed to be specific for ICSH activity.

For construction of dose-response curves for male gonadotropin and for cyclic and post-menopausal female gonadotropins, both tests were used (Figs. 1 and 2). Both tests were also employed in 4-point assays in which the various gonadotropins were compared with HMG-20 (Figs. 3–5). For male and for cyclic female gonadotropins two such assays were conducted on each type of urine extract; one assay was performed on post-menopausal urine. The recovery experiments involving pooled male and cyclic female

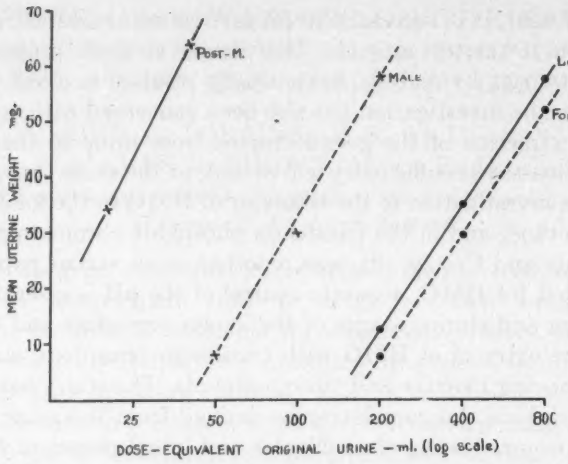

FIG. 1. Dose-response curves for urinary gonadotropins from post-menopausal women, from normal men and from cyclic women (luteal and follicular phases). (Uterine weight test—intact mice).

urine extracts were performed in duplicate, and in these estimations the mouse uterus test alone was used (Figs. 6 and 7). The assay methods are described in a previous paper by Loraine and Brown (10).

(iii) *Statistical methods*

(a) *Design of assays.* Fourteen assays were performed in which the various types of gonadotropins were compared with HMG-20 (Tables 1 and 2). In 7 of these the end-point

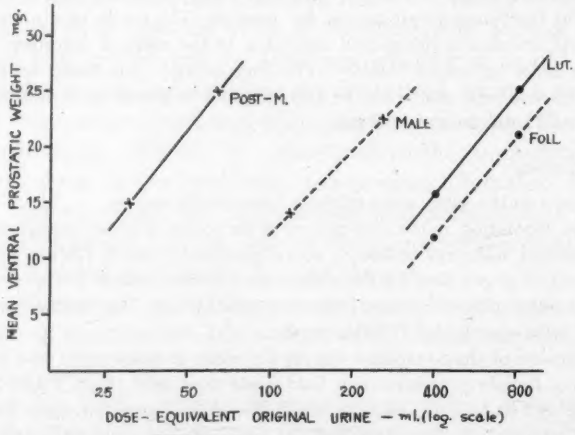

FIG. 2. Dose-response curves for urinary gonadotropins from post-menopausal women, from normal men and from cyclic women (luteal and follicular phases). (Ventral prostatic weight test—hypophysectomized rats).

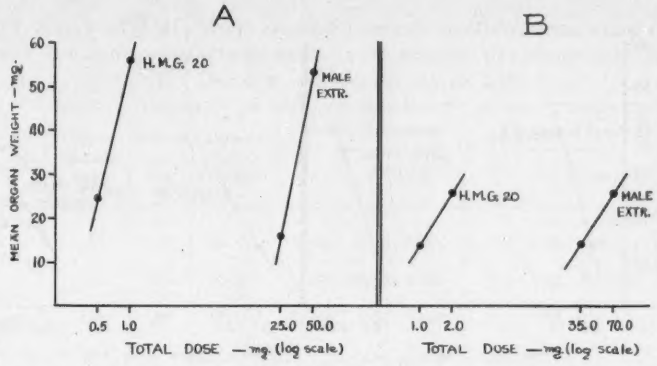

FIG. 3. Four-point assays of male gonadotropin against HMG-20. **A** = Uterine weight test (intact mice). **B** = Ventral prostatic weight test (hypophysectomized rats).

depended on enlargement of the uterus in intact immature mice, and in 7 on enlargement of the ventral lobe of the prostate in hypophysectomized immature rats. In 13 instances the design was a 4-point assay with two dose levels of the standard (S) and unknown (U) preparations, and with equal spacing of the log doses. In one assay in mice a 6-point design (3 doses of S versus 3 doses of U) was employed. The number of animals per dose level of S and of U varied from 3 to 7 but was most frequently 5. The log dose interval, *i.e.*, the difference between the logarithms of the two doses, was equal to $\log_{10}^{2}$ (0.301). The mathematical basis of the 4-point assay and the methods of calculation used in the present study have been described in papers by Emmens (14), Finney (15), Gaddum (16, 17), and Diczfalusy and Loraine (18).

(b) *Validity tests.* These were performed according to the techniques recommended by Gaddum (16, 17). As all but one of the assays had a 4-point design, it was possible to test for parallelism but not for curvature. The index "G" is a measure of the difference between two slopes. The ratio of "G" to its standard deviation furnishes a value for

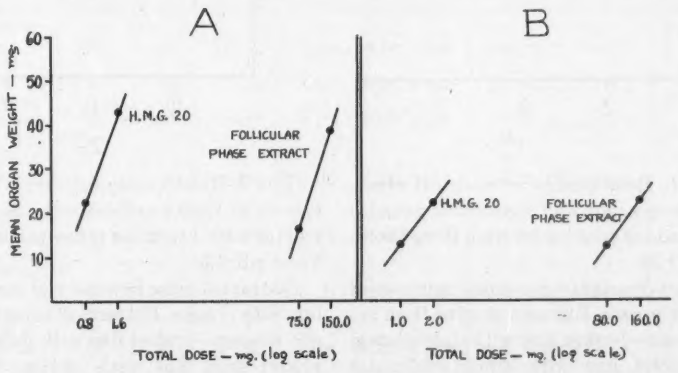

FIG. 4. Four-point assays of follicular-phase gonadotropin against HMG-20. **A** = Uterine weight test (intact mice). **B** = Ventral prostatic weight test (hypophysectomized rats).

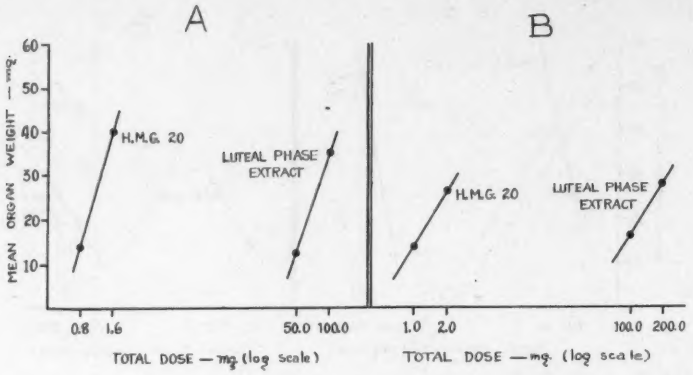

FIG. 5. Four-point assays of luteal-phase gonadotropin against HMG-20. **A** = Uterine weight test (intact mice). **B** = Ventral prostatic weight test (hypophysectomized rats).

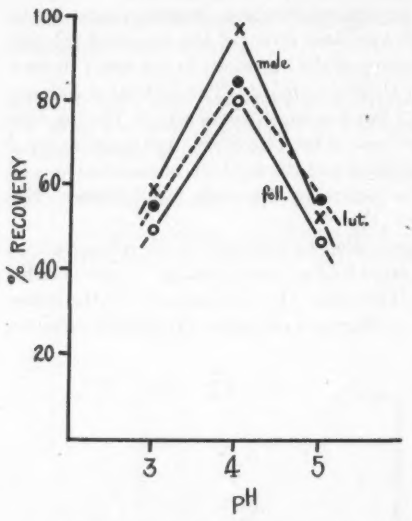

FIG. 6. Relationship between pH of adsorption on kaolin and recovery of gonadotropins added to urine (elution from kaolin at pH 11.3).

Extract of urine from normal men = solid line with crosses. Extracts of urine from cyclic women—broken line with dots = luteal phase; solid line with circles = follicular phase.

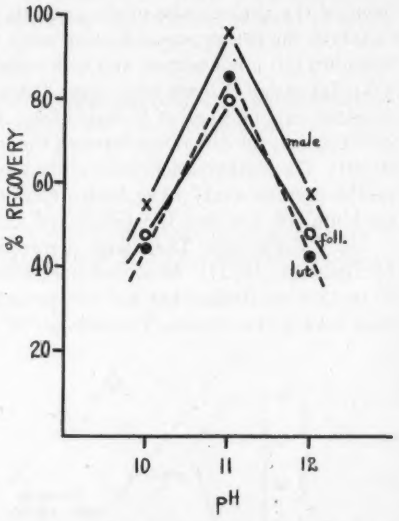

FIG. 7. Relationship between pH of elution from kaolin and recovery of gonadotropins added to urine (adsorption on kaolin at pH 4.0).

Extract of urine from normal men = solid line with crosses. Extracts of urine from cyclic women—broken line with dots = luteal phase; solid line with circles = follicular phase.

TABLE 1. ASSAY OF HMG-20 (S) AGAINST URINARY GONADOTROPINS FROM NORMAL MEN AND FROM NORMALLY MENSTRUATING WOMEN (U)—MOUSE UTERUS TEST (4-POINT ASSAYS: DOSE RATIO, 2.0)

Materials administered	No. of animals/ assay	Potency of U%	Fiducial limits % (P = 0.95)	Indices of precision			Parallelism data			Ratio of activities S/U
				λ	L	g	G	t(G)	P(G)	
HMG-20 *vs.* Male extract	18	93	76–113	0.089	11.38	0.070	0.6	0.07	0.9	54
HMG-20 *vs.* Male extract	18	104	86–127	0.098	10.20	0.094	2.9	0.31	0.7–0.8	36
HMG-20 *vs.* Follicular-phase extract	15	88	44–134	0.168	5.97	0.340	11.6	0.79	0.4–0.5	114
HMG-20 *vs.* Follicular-phase extract	16	86	66–103	0.084	10.90	0.083	1.5	0.20	0.7–0.8	110
HMG-20* *vs.* Luteal-phase extract	20	40	14– 63	0.216	4.64	0.166	2.0	0.48	0.6–0.7	125
HMG-20 *vs.* Luteal-phase extract	16	91	70–114	0.097	10.28	0.114	3.8	0.48	0.6–0.7	68
HMG-20 *vs.* Post-menopausal extract	15	100	81–124	0.078	12.80	0.070	2.0	0.31	0.7–0.8	3.0

* Six-point assay.

TABLE 2. ASSAY OF HMG-20 (S) AGAINST URINARY GONADOTROPINS FROM NORMAL MEN AND FROM NORMALLY MENSTRUATING WOMEN (U)—VENTRAL PROSTATE TEST IN HYPOPHYSECTOMIZED RATS (4-POINT ASSAYS: DOSE RATIO, 2.0)

Materials administered	No. of animals/ assay	Potency of U%	Fiducial limits % (P = 0.95)	Indices of precision			Parallelism data			Ratio of activities S/U
				λ	L	g	G	t(G)	P(G)	
HMG-20 *vs.* Male extract	18	92	71–114	0.114	8.72	0.133	2.6	0.70	0.4–0.5	33
HMG-20 *vs.* Male extract	14	98	71–126	0.097	10.34	0.133	1.2	0.31	0.7–0.8	36
HMG-20 *vs.* Follicular-phase extract	20	101	82–137	0.119	8.44	0.127	0.7	0.25	0.8–0.9	80
HMG-20 *vs.* Follicular-phase extract	19	83	58–112	0.109	9.19	0.113	1.1	0.42	0.6–0.7	91
HMG-20 *vs.* Luteal-phase extract	17	115	96–139	0.078	12.70	0.068	0.9	0.32	0.7–0.8	87
HMG-20 *vs.* Luteal-phase extract	21	95	65–116	0.131	7.62	0.148	1.6	0.55	0.5–0.6	80
HMG-20 *vs.* Post-menopausal extract	22	97	78–121	0.098	10.25	0.076	0.7	0.32	0.7–0.8	2.8

"t," the significance of which can be tested by reference to an appropriate table. A significant value for "G" can be interpreted as evidence that the log dose effect curves are not parallel. If the value for "G" is not significant the assumption is that, although the curves are not exactly parallel, the difference in the slopes is no greater than might reasonably have been expected.

(c) *Indices of precision.* Estimations were made of:

1. *Gaddum's* "λ", which is equal to the standard deviation of the logarithm of the individual effective dose (19, 20). This quantity is independent of the units of measurement and of the experimental design. For assays depending on graded responses, the ratio of the standard deviation of the response to the slope of the regression line (s/b) yields an estimate of "λ."

2. *Woolf's* "*L*," which is the reciprocal of "λ." It has recently been claimed (21) that in assays depending on graded responses this quantity should be used instead of "λ," since when the total number of observations in each experiment is large, the distribution of "L" is approximately normal with variance $1/S\ (x-\bar{x})^2$. Gaddum (16) has suggested that in addition to other indices of precision, such as "λ" or "g," different assay techniques should be compared in terms of their mean value of "L."

The weighted mean value for "λ" in the two assay methods used was estimated from the expression $\bar{s}/\bar{b}$, where $\bar{s}$ is the square root of the weighted error mean square computed from all the experiments using the technique: $b=S(wb)/S(w)$ and $w=1/V(b)$ in each assay. The method of calculation has been recently described by Diczfalusy (22).

3. *Finney's* "*g*," which is an estimate of the regression coefficient and furnishes an indication of the certainty with which the regression coefficient "b" is estimated (15, 23). For an accurate assay, "g" is 0.1 or less. A value of unity or more indicates, on the other hand, that there is no real regression and that the assay is therefore invalid.

Calculations of the relative potency (R) and of the fiducial limits were made according to the formulae described respectively by Gaddum (19) and by Finney (15).

B. Chemical

The kaolin used was acid washed (Society of Leather Trades Chemist's specification) and was supplied by British Drug Houses Ltd. The tricalcium phosphate suspension was prepared according to the method of Swingle and Tiselius (24) and contained approximately 0.07 Gm. of $Ca_3(PO_4)_2$ per ml. Hydrogen ion concentrations were measured by means of a Pye Universal pH meter using a lithium glass electrode.

(i) *Preparation of crude male, follicular-phase female, luteal-phase female and menopausal gonadotropin*

Pooled urine was collected from normal male subjects and from normally menstruating women between the fifth and twelfth days (follicular phase) and between the eighteenth and twenty-eighth day (luteal phase) of their cycles. Two samples of post-menopausal urine were collected. The first (Sample "A") which was used for construction of the dose-response curves was a pooled specimen obtained from 4 patients suffering from non-endocrine diseases; the second (Sample "B"), which was used for comparison with HMG-20, was collected from a single post-menopausal subject aged 55.

The urine was adjusted to pH 4.0 with HCl. A suspension of kaolin (10 Gm. in 50 ml. of water) was added to each liter of original urine, the pH was rechecked and corrected if necessary, and the mixture was stirred mechanically for one hour. The suspension was centrifuged and the kaolin deposit was suspended in water (25 ml. per liter of original urine) and adjusted to a pH of 11.3 with NaOH.

The suspension mixture was allowed to stand for twenty minutes, with occasional shaking, and was then centrifuged. The pH of the supernatant fluid was adjusted to 4.0–6.0 with HCl and the gonadotropins were precipitated with 5 volumes of acetone. The precipitate was collected, washed with absolute ethanol and ether, then dried over calcium chloride.

The yield of crude material from 53 liters of pooled male urine was 15.6 Gm.; from 54 liters of follicular-phase urine, 15.5 Gm.; and from 100 liters of luteal-phase urine, 28.8 Gm. For post-menopausal urine, 4 liters of Sample "A" and 2.6 liters of Sample "B" yielded respectively 1.0 Gm. and 0.5 Gm. of crude material.

At the dosage levels required to elicit a biologic response, the kaolin-acetone powders from post-menopausal urine were tolerated by both mice and rats. Those from follicular and luteal-phase urines were generally toxic to all test animals, and those from male urine were tolerated by intact mice but not by hypophysectomized rats. Accordingly, in the case of male and cyclic female gonadotropins it was necessary to purify the extracts further before using them in assays against HMG-20. The procedure developed is as follows:

(ii) *The purification of crude kaolin-acetone powders*

The method depends on the fact that the gonadotropins themselves are very soluble in water, whereas the main bulk of inactive material is relatively insoluble. However, extraction of the gonadotropins from the crude powder with water is usually incomplete unless large volumes are used, probably because the gonadotropins are partly adsorbed onto the inactive material at the pH of the resulting mixture (usually about pH 6.0). The degree of the adsorption depends on the pH at which the extraction is performed and is considerably decreased by increasing the pH. Unfortunately this also increases the solubility of the inactive material. A satisfactory compromise between these two factors was reached by working at pH 8.0–8.5. Under these conditions, providing certain minimum requirements of water volume to powder weight were satisfied, all the activity could be extracted with water and a large proportion of the inactive material, being undissolved, could be discarded. The extracts thus obtained are then further purified by treatment with tricalcium phosphate by the method described for HMG in the earlier paper (10). If necessary, further concentration of the extracts can be achieved by precipitating with 5 volumes of acetone at pH 4.0–5.0, and eluting the dried precipitate again with water at pH 8.0. Before bioassay, all extracts are adjusted to pH 5.0–5.5 and any deposit formed is separated off by centrifugation.

The method. Kaolin-acetone powder (1 Gm.) was ground with water (20 ml.) and the pH was adjusted to 8.0–8.5. The mixture was stirred for twenty minutes, then centrifuged. The deposit was similarly extracted with a further volume of water (5 ml.). The aqueous extracts were combined and tricalcium phosphate suspension (4 ml.) was added and the pH adjusted to 8.0–8.5. The whole was stirred for fifteen minutes, then centrifuged, and the supernatant fluid containing the gonadotropins was removed. At the same time, the insoluble residue from the kaolin-acetone powder was extracted again with water (5 ml.). This extract was stirred with the tricalcium phosphate deposit in order to remove any remaining gonadotropin. The resulting suspension was centrifuged and the supernatant fluid was added to the gonadotropin solution. The pH of this was adjusted to 5.0 (approx.) with HCl, acetone (5 vols.) was added, and the whole allowed to stand in the refrigerator overnight. The precipitate was collected, washed with absolute ethanol and ether, and then dried over calcium chloride.

In preparing the resulting powder for injection, the volume of water used depended

on the final concentration of solution required for injection. The following proportions are for a final volume of 10 ml. The acetone-precipitated powder was ground with water (5 ml.) and the pH adjusted to 8.0–8.5. The mixture was stirred for twenty minutes, then centrifuged. The deposit was similarly extracted with two further amounts of water (2.5 ml.). The aqueous extracts were combined, adjusted to pH 5.0–5.5, and placed in the refrigerator overnight at 4° C. On the following day any precipitate was centrifuged off, the volume was adjusted to 10 ml., and the clear, pigmented supernatant fluid was injected into the test animals.

(iii) *The behavior of male and cyclic female gonadotropins in the kaolin-acetone and tricalcium phosphate procedures*

The experiments for determining the behavior of male and cyclic female gonadotropins in the kaolin-acetone and tricalcium phosphate procedures were limited by the shortage of materials but were similar in design to those described for HMG and HCG in the preceding paper (10). The crude luteal-phase or follicular-phase gonadotropin extract (4.0 Gm.) or the crude male gonadotropin extract (1.25 Gm.) was dissolved in pooled male urine (250 ml.) at pH 8.0. The insoluble material was centrifuged off and the supernatant fluid divided into five 50-ml. fractions. The pH of each was adjusted with HCl as follows: #1—3.0; #2, #3 and #4—4.0; #5—5.0. Kaolin (1 Gm.) was added to each and stirred to form an even suspension; the pH's were rechecked and adjusted if necessary; and the suspensions were stirred for twenty-five minutes and centrifuged. The kaolin deposits were suspended in water (10 ml.) and the pH was adjusted with NaOH as follows: #1, #3 and #5—11.3; #2—10.0; #4—12.0. The suspensions were allowed to stand twenty minutes, with occasional shaking, and were then centrifuged. The supernatant fluids were treated with tricalcium phosphate suspension (2 ml.) at pH 8.0. The tricalcium phosphate was centrifuged off, washed with water (2 ml.), the supernatant fluids plus the water washes were adjusted to pH 5.0, and acetone (5 vol.) was added. The precipitates were collected and dried. The resulting powders were extracted once with 2.5 ml. of water at pH 8.0–8.5 and twice with 1.5 ml. of water. The extracts were adjusted to a pH of 5.0–5.5 and a volume of 5 ml. and centrifuged. The activity present was compared with that in the equivalent weight of original crude powder, purified as described in the preceding section.

In order to ascertain whether the crude kaolin-acetone powders from the urines of males and of cyclic females contained activity with the properties of the "GB" fraction (25), the tricalcium phosphate deposits from each of the foregoing series of experiments were combined and eluted with water (10 ml.) at pH 10.5. The eluate was concentrated by precipitating with acetone (5 vol.) at pH 5.0 and by dissolving the dried precipitate in water (5 ml.). The final extract was then tested for gonadotropic activity, using the mouse uterus test. No activity was detected in a dose equivalent to five times that of the "GA" fraction. It can therefore be concluded that these gonadotropins resemble HMG in not being adsorbed on tricalcium phosphate at pH 8.

RESULTS

A. Biologic

(i) *Dose-response curves for male, post-menopausal female and cyclic female gonadotropins*

The curves for the mouse uterus test are shown in Figure 1 and for the rat prostate test in Figure 2. It will be noted (Fig. 1) that over the dosage

range used there was a linear relationship between the log-dose and the effect. By both tests post-menopausal urine was approximately five times more active than pooled male urine and male urine was approximately four times more active than pooled follicular-phase and luteal-phase urine.

(ii) *Assays of male, cyclic female and post-menopausal gonadotropins against HMG-20*

The results are shown in Figures 3, 4 and 5 and in Tables 1 and 2. In Figures 3–5 the dosages of urine extracts from males and cyclic females are expressed in mg. of crude material prepared by the kaolin-acetone method. Table 1 presents statistical data in 6 assays in which the various types of gonadotropin were compared with HMG-20, using the mouse uterus test. In one of the assays on male urine and in the assay on post-menopausal urine, crude kaolin-acetone powders were employed. In the other assay on male urine and in all assays involving follicular-phase and luteal-phase urines the crude material was treated with tricalcium phosphate as previously described. The weighted mean index of precision (λ_w) was 0.110 and the weighted mean "L" was 9.11. It will be noted that in all assays the value for "G" was not significant. It can therefore be assumed that there was no significant difference in the slopes of the various dose-effect curves and that HMG-20 can be used as a standard preparation for the assay of male, cyclic female and post-menopausal female gonadotropins.

Table 2 shows the results obtained when the same urine extracts were compared with HMG-20, using the ventral prostatic weight test in hypophysectomized immature rats. In all assays involving male and cyclic female gonadotropins the crude material was purified as described, in order to reduce toxicity. The weighted mean index of precision (λ_w) was 0.105 and the weighted mean "L" was 9.50. Again it was noted that in no case was the value for "G" significant, indicating that the slopes of the various log dose-effect curves did not differ significantly.

In Table 3 the activity of HMG-20 is compared with that of the other urinary gonadotropins in both assay methods. With the mouse uterus test the mean ratio of activities (standard/unknown; S/U) for male urine was 45, for follicular-phase female urine 112, and for luteal-phase female urine 97. The corresponding figures, using the rat prostate test, were respectively 35, 86 and 84. In the single assay conducted on post-menopausal urine the ratios were 3.0 for the mouse uterus test and 2.8 for the rat prostate test. It will be seen that there was no significant difference between the estimations obtained by the two assay methods for the relative potency of the 4 different gonadotropic extracts.

Gaddum (26) recently suggested that results of this type can be expressed in terms of the "*index of discrimination.*" This is equal to the ratio of the two results which would be obtained if one of the substances

TABLE 3. COMPARISON OF URINARY GONADOTROPINS FROM MALES AND FROM NORMALLY MENSTRUATING WOMEN WITH HMG-20, USING DIFFERENT ASSAY METHODS

Urinary extract administered	Ratio of activities (standard/unknown), uterine wt. test, mice	Ratio of activities (standard/unknown), vent. prostatic wt. test, rats	Mean index of discrimination (standard/unknown), uterus/ventral prostate
Male	54	33	
	36	36	1.30
Follicular-phase female	114	80	
	110	91	1.31
Luteal-phase female	125	87	
	68	80	1.16
Post-menopausal female	3.0	2.8	1.07

were used to assay an unknown extract containing the other. The values obtained in the present investigation are shown in the last column of Table 3. It will be seen that with all 4 types of urinary extract the index was approximately equal to unity. This suggests that the active principle measured by the two tests is identical and provides further evidence that there is no qualitative difference between the various urinary gonadotropins in non-pregnant subjects when these substances are extracted from urine by the kaolin-acetone method.

B. Chemical

(i) *Kaolin-acetone procedure*

The results of recovery experiments with male and cyclic female gonadotropins are presented in Figures 6 and 7. All assays were conducted by the mouse uterus test and each point represents the mean of at least two such experiments. Figure 6 shows that maximal adsorption of male and cyclic female gonadotropins onto kaolin occurs at pH 4.0, and Figure 7 shows that the optimal pH for eluting these substances from kaolin is between 11.0 and 11.5. The pH's of both adsorption and elution are critical. It will be noted that the behavior of these gonadotropins with respect to kaolin adsorption and elution is very similar to that previously observed for HMG (10).

(ii) *Tricalcium phosphate adsorption*

By treatment of crude kaolin-acetone powders with tricalcium phosphate according to the method described, it is possible to administer the equivalent of approximately 1 liter of original urine per animal. It was of importance to determine whether this purification step resulted in any loss of gonadotropic activity. Accordingly, two 4-point assays were conducted in which the activity of a crude kaolin-acetone extract from urine of a normal man was compared with the purified material from the same source. The mouse uterus test was used, with 5 animals per dose level of crude and purified extracts. The crude extract was injected as a thick suspension, whereas the purified material was administered as a clear solution. The mean index of precision (λ) was 0.125 and the mean value for "L", 9.05. In neither assay was the value for t(G) significant, indicating that there was no significant difference in the slopes of the log dose effect curves. The mean ratio of activities (purified/crude extract) was 1.22. The slightly greater activity of the purified material is probably attributable to the fact that the crude extract proved somewhat toxic to the animals, especially at the higher dosage levels.

It can therefore be concluded that in the case of urine from normal men the purification step does not cause any loss of gonadotropic activity. Similar experiments with urine from cyclic females could not be performed because the crude material was generally toxic to mice in a dosage necessary to elicit a biologic response.

DISCUSSION

The present investigation shows that it is possible to use a gonadotropin extract of urine from human menopausal and post-menopausal subjects (HMG), prepared by the kaolin-acetone method, as a standard preparation for the assay of similarly prepared gonadotropin extracts of urine from non-pregnant subjects. The introduction of a preparation such as HMG-20 as a universal standard for these assays should increase considerably the reliability of the determinations. It should also make it possible to compare results from different laboratories, and to eliminate the need for expressing results in terms of "animal units." When using HMG as a standard preparation, the statistical design of the assay will be determined essentially by the variability of the strain of animals employed. In this laboratory the mouse uterus test is the method usually adopted for routine clinical estimations of gonadotropins. A 2+1 or 2+2 assay design is used with 5 animals per dose level of standard and unknown preparations. The value for the index of precision (λ) is generally less than 0.2, and this would appear to be a satisfactory degree of precision for quantitative work.

The test depending on the enlargement of the prostate in hypophysectomized immature rats is generally assumed to be specific for interstitial-cell stimulating hormone (ICSH) activity. The method is reasonably accurate and is sufficiently sensitive to measure urinary ICSH in clinical conditions in man (10, 13). Although the technique is too tedious and laborious for routine studies it is probable that valuable information will be obtained by its use in selected patients.

Methods of extraction of gonadotropins from the urine of human non-pregnant subjects have always suffered from the disadvantage that the final extracts obtained are often toxic to the experimental animals. The present investigation has shown that by eluting the crude kaolin-acetone powders with water under controlled conditions and treating the eluate with tricalcium phosphate it is possible to administer to intact mice and hypophysectomized rats gonadotropin extracts equivalent to approximately 1 liter of the original urine. The indications are that practically all gonadotropic activity present in the original powder is recovered by this purification procedure. Proof of this could be obtained only in the case of extracts from the urines of men and post-menopausal women, in which no loss of activity was observed when extracts were assayed by the mouse uterus test. In assays employing hypophysectomized rats, crude kaolin-acetone powders are generally toxic and it is necessary to employ the purification procedures routinely. In assays conducted on intact immature mice, such powders are well tolerated when the gonadotropin excretion is high, *e.g.*, in menopausal and post-menopausal subjects; in low-titer urines, however (*e.g.*, in normally menstruating women), it is generally advisable to incorporate the purification steps in the extraction procedure.

The recovery experiments conducted with gonadotropin extracts from the urines of normal men and of menstruating women showed that these substances behaved in a manner very similar to HMG in both the kaolin-acetone and tricalcium phosphate procedures (Figs. 6 and 7). As with HMG, the critical pH for adsorption was 4.0 and for elution, 11.0–11.5. It should be emphasized, however, that these estimations were made with gonadotropic material which had already been extracted from urine by the kaolin-acetone method at these optimum pH figures. Further work is needed to show whether these results also apply to the gonadotropins present in the original urine.

There is now good evidence, using hypophysectomized rats, that both follicle-stimulating and luteinizing activities are present in the urine of normal men, and of cyclic and post-menopausal women (3, 10, 11, 13, 27–32). Most workers have assumed that human urine resembles pituitary tissue from sheep and swine in containing two separate substances, FSH and ICSH. However it has not so far been possible to separate human urinary

FSH from human urinary ICSH and it is not yet clear whether the effects are due to two substances or to a single substance with both activities. The latter view has been favored by Segaloff *et al.* (33). On the other hand Walter (34), using the mouse uterus and ventral prostatic weight tests, has demonstrated that extracts of post-menopausal urine prepared by the tannic-acid method may differ qualitatively from HMG-20. In the present study, in which the same two bioassay methods were employed, there was no significant difference between the estimates obtained by the two methods for the relative potency of the 4 different gonadotropin extracts. This was indicated by a mean index of discrimination of approximately unity in all cases (Table 3). This finding suggests that there is little qualitative difference between the various gonadotropins from non-pregnant subjects, when the extracts are prepared from urine by the kaolin-acetone method.

SUMMARY

1. Urinary gonadotropins from normal men and from normally menstruating and post-menopausal women can be assayed in terms of Human Menopausal Gonadotropin (HMG), using assay methods depending on the enlargement of the uterus in intact immature mice and enlargement of the prostate in hypophysectomized immature rats. It is proposed that a preparation of HMG be used as a standard for the assay of urinary gonadotropin in non-pregnant human subjects.

2. By both bioassay methods, urine from post-menopausal women was approximately five times more active than urine from normal men, and the latter urine was approximately four times more active than follicular-phase or luteal-phase urine.

3. Male, and both follicular-phase and luteal-phase female gonadotropins behave like HMG in the kaolin-acetone and tricalcium phosphate procedures.

4. A purification procedure for crude kaolin-acetone powders is described, which makes it possible to administer to intact mice and to hypophysectomized rats the equivalent of approximately 1 liter of the original urine.

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VASCULAR REACTIVITY AND ELECTROLYTES IN NORMAL AND TOXEMIC PREGNANCY.

PATHOGENIC CONSIDERATIONS AND A DIAGNOSTIC PRE-TOXEMIA TEST*

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FOR decades, the origin of toxemia of pregnancy was the subject of many more or less poorly founded speculations. Hofbauer (1) was probably the first to suspect an important involvement of the adrenal cortex in its pathogenesis. Finally, the demonstration of an increased corticoid production during pregnancy and of the role played by adrenal corticoids in the formation of edema and in the pathogenesis of arterial hypertension provided some solid footholds from which to attack the problem systematically.

Recent investigations (cited (2)) have shown that the tonus of the arterial walls is in general determined by their intramural catechol-amine content (norepinephrine from the local sympathetic nerve endings and epinephrine from the adrenal medulla), and by the adrenal corticoids which make the vascular cells responsive to pressor catechol-amine action. The latter conclusion is based on the observation, made by one of us (3, 4) and later by others (5, 6, 7[1]), that the pressor effect of injected or infused norepinephrine and epinephrine is significantly intensified by pre-treatment with desoxycorticosterone acetate (DCA). Adrenocortical extracts (8, 9) and cortisone (10, 11) were likewise found to sensitize the vascular system to the catechol amines. By contrast, the pressor effects of epinephrine and norepinephrine are weakened after adrenalectomy (9, 12, 13).

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[1] These authors did not place much emphasis on the effect of desoxycorticosterone acetate in augmenting the pressor action of epinephrine, but statistical evaluation of their data by Vanatta and Cottel (6) showed the augmentation to be significant.

The mechanism by which the "glucocorticoids" enhance vascular reactivity to the pressor catechol amines is not clearly understood but it may be assumed that interferences in cellular carbohydrate metabolism are involved. As far as the action of "mineralocorticoids" (experimentally represented by DCA) is concerned, it is known that they augment the intracellular sodium concentration of cardiovascular tissue (14–16) and thus alter its intra-extracellular cationic gradient. Since this gradient determines the electrical cell-membrane potential (17) and through it the contractile amplitude of the muscular cells in response to depolarizing stimuli (17, 18), it is believed that the "mineralocorticoids" sensitize the arterial walls to the depolarizing action of the catechol amines, chiefly by means of their tendency to deposit sodium inside the contractile cells (19, 20). Sodium deprivation on the other hand, reduces the sodium content of the arterial walls (21) and was found by us (22) to weaken, or even to abolish, both the sensitizing action of DCA and the pressor effectiveness of norepinephrine and epinephrine per se.

The foregoing considerations suggested the possibility that an abnormally increased corticoid activity, by potentiating the intrinsic action of catechol amines on the vascular system, might play a prominent role in the pathogenesis of toxemic hypertension, comparable to that in Cushing's disease, in the "endocrine" type of essential hypertension described by Schroeder (23), and in the "primary aldosteronism" described by Conn (24).

A marked increase of total corticoid and 17-hydrocorticosteroid production and excretion occurs as a characteristic feature of the third trimester of pregnancy (25–27), reaching its peak between day 200 and day 240 of gestation (25). It is suspected to be largely of placental origin (28–32) and to include aldosterone from the placenta (33). Significantly, the curve of corticoid production runs nearly parallel with that of pregnancy-induced hypervolemia, and with that of the statistical incidence of cardiac complications (25, 34).

In cases of toxemia, the amount of total urinary corticoids was found either unaltered (35–37) or moderately increased (38). However, a marked abnormality in the qualitative composition of the corticoid spectrum was demonstrated by Venning *et al.* (35), in that the "mineralocorticoid" fraction appeared augmented at the expense of the" glucocorticoids." An abnormal increase of reducing (39, 40), purely water-soluble (30, 41) and sodium-retaining (42) corticoids, notably aldosterone (43, 44) was likewise observed to precede and to accompany the toxemic syndrome and to disappear with the onset of clinical improvement (39). This has been attributed to a disturbance in placental corticoid formation and metabolism (30, 32). A fetal origin seems unlikely, since the newborn infant produces

only minimal amounts of corticoids and is resistant to corticotropin stimulation (45). Experimentally, a toxemia-like syndrome has been elicited in animals through the administration of DCA combined with a high sodium intake (46, 47).

Aside from the well known sodium retention in toxemia of pregnancy (48), an abnormal tendency toward intracellular sodium accumulation, analogous to that resulting from DCA administration (14–16; 49–51), has been noted in cases of toxemia (52, 53). It has also been noted that clinical improvement was accompanied by a decrease of intracellular sodium and increase of potassium (53).

In consideration of the described potentiating effect of mineralocorticoids upon pressor catechol-amine action, and in view of existing evidence in favor of an augmented mineralocorticoid production preceding and during toxemia of pregnancy, we studied the pressor response to infused norepinephrine and epinephrine in 163 normotensive pregnant women, including a group showing pre-hypertensive pre-toxemia and toxemia. The controls were 60 nulliparous normal young women.

METHODS

With very few exceptions, the tests in the pregnant women (average age 24.5 years) were carried out between the twenty-eighth and thirty-fourth week of pregnancy, the period of maximal physiologic corticoid production (25).

Because of several difficulties which had to be overcome in making the women keep their appointments for the tests, we could not insist on a uniform time of the day nor on the fasting state, but as a rule, at least two hours had elapsed between the patient's last meal and the performance of the test.

Sodium and potassium levels were determined in both serum and saliva. First, saliva was collected according to the procedure recommended by Frawley and Thorn (54). After the patients had voided, blood was withdrawn from the cubital vein through a cannula and two three-way stopcocks, connected with each other and with three 500-cc. saline infusion bottles, were attached to the cannula. One bottle contained physiologic saline solution. In the two other bottles, 2 cc. of an epinephrine solution (adrenalin 1:1000—Parke, Davis & Co.) and 2 cc. of *l*-norepinephrine solution (Levophed 1:1000—Winthrop Laboratories), respectively, had been added to the saline, so that each bottle contained 4 micrograms per cc. of the respective catechol amine.

In order to obtain an accurate infusion dosage, it was necessary to calibrate each of the Venopak venoclysis units with which the bottles containing the catechol amines were equipped. This was achieved by counting the drops of fluid needed to fill the space of 10 cc. in a test tube. The number of drops per 10 cc. was multiplied by the weight of the test subject in kilograms and divided by 400, to determine the number of drops for the dosage of 0.1 microgram per kilogram per minute. This number was then multiplied by 2 or 3, in accordance with the desired dosages of 0.2 or 0.3 μg./Kg./min. respectively, and an electric metronome was set for the calculated number of drops per minute.

At the beginning of the test, physiologic saline was infused into the reclining patient at the rate of about 60 drops per minute until a stable minimal blood pressure level was established. Then, without the knowledge of the test subject, the connection of the

cannula was switched from plain saline to norepinephrine by exchanging the clamps on the tubings. The drop count, yielding 0.2 μg./Kg./min. was synchronized with the metronome by adjusting the screw of a pinchcock which was fastened on the Venopak tubing.

Blood pressure readings were recorded immediately before infusion and at the end of each minute during a five-minute infusion period. At the end of the fifth minute, the inflow was switched back to plain saline. As soon as the blood pressure had returned to the pre-infusion level and persisted there for about ten minutes, the same procedure was repeated with 0.3 μg./Kg./min. of *l*-norepinephrine, and subsequently with 0.2 and 0.3 μg./Kg./min. of epinephrine.

Each complete set of four infusions, together with the collection of saliva, required approximately two hours. In a few instances, only the smaller epinephrine dose was given, and even that was stopped if the test subjects complained of palpitation or headache. Side-effects of norepinephrine were minimal or nil, despite higher elevations of the blood pressure.

Electrocardiograms were taken in all cases before and during the infusions. The results will be published separately.

RESULTS

Blood pressure

The absolute blood pressure deviations caused by norepinephrine and epinephrine in 60 nulliparous female control subjects (average age 23 years) were practically identical with those previously observed by us (4) in 15 physically healthy males (average age 36.5 years), even though the average initial resting levels of blood pressure in the latter group were 30/20 mm. higher.

Five of the female control subjects in whom the most pronounced reactions had occurred were re-examined within two years. There was no significant change in either the resting blood pressure level or in the pressor responses to the infusions. The constancy of reactivity was thereby illustrated.

Two control subjects became pregnant and were re-examined during the third trimester of pregnancy. No major changes in the pressor reactions were noted.

The resting blood pressure levels and the average pressor responses in 100 normal pregnancy cases (no edema, no albuminuria, no hypertension)were nearly identical with those in the 60 controls (Table 1; Figs. 1 and 2).

The average resting blood pressure levels and the average pressor responses of the 27 oldest pregnant women (age 31 years or over) did not differ significantly from those of the 30 youngest women (age 19 years or under) (Table 2A). Average resting blood pressure and average pressor responses were likewise practically identical in 47 primiparae and in 40 multiparae who had gone through four or more preceding pregnancies (Table 2B).

Thirty-three pregnant women were re-examined when in the non-preg-

TABLE 1. BLOOD PRESSURE RESPONSES TO CATECHOL AMINES IN NULLIPAROUS CONTROLS AND IN NORMAL PREGNANT WOMEN

	No. of cases	Age (avge.)	Weight (lbs.) (avge.)	Basal bl. pressure (mm.Hg) (avge.)	Blood pressure deviations from basal level (averages)			
					Systolic	Diastolic	Systolic	Diastolic
					Norepinephrine			
					0.2 μg./Kg./min.		0.3 μg./Kg./min.	
Nulliparous	60	23	126	106/61	+18.2 (± 9.6)*	+16.5 (± 6.9)	+27.6 (±12.3)	+24.1† (± 7.9)
Pregnant (normal)	100	25	150	108/60	+19.5 (± 9.4)	+15.7 (± 7.1)	+29.3 (±12.4)	+21.4 (± 9.5)
					Epinephrine			
					0.2 μg./Kg./min.		0.3 μg./Kg./min.	
Nulliparous	60	as above		106/61	+18.2 (± 6.5)	− 6.5‡ (± 7.7)	+27.1‡ (± 9.4)	+ 3.3† (±10.4)
Pregnant (normal)	99	as above		108/60	±16.0 (± 8.9)	− 2.6 (± 8.8)	+22.5 (±10.8)	+ 0.6 (± 8.9)

* The figures in parentheses indicate the standard deviations.
† Difference from the normal pregnancy group significant at the 2–5 per cent probability level (*t*-test, Fisher).
‡ Difference from the normal pregnancy group significant at the 1 per cent probability level.

TABLE 2. INFLUENCE OF VARIOUS FACTORS (A, B AND C) ON THE BLOOD PRESSURE RESPONSE TO CATECHOL AMINES

		No. of cases	Age (avge.)	Weight (lbs.) (avge.)	No. of pregnancies (avge.)	Basal bl. pressure (mm. Hg) (avge.)	Blood pressure deviations from basal level (averages)			
							Norepinephrine		*Epinephrine*	
							0.2 μg./Kg./min.	0.3 μg./Kg./min.	0.2 μg./Kg./min.	0.3 μg./Kg./min.
A	Age 19 or less	30	18	136	1	111/61	+21/+16	+31/+22	+16/−5	+26/−3
	Age 31 or more	27	35	145	5	108/63	+26/+16	+37/+21	+19/+1	+28/+3
B	Primiparae	47	22	140	1	111/62	+23/+16	+35/+20	+18/−2	+25/+1
	Multiparae (5 or more pregnancies)	40	29	151	6	110/63	+21/+16	+30/+22	+19/±0	+28/+3
C	During pregnancy	33	26	144	4	107/58	+24/+18	+36/+24	+17/−2	+25/−1
	Same women after pregnancy	33	27	125	—	114/69	+28/+19	+42/+29	+20/±0	+32/+7

A = Influence of age.
B = Influence of number of pregnancies.
C = Influence of pregnancy. Repetition of tests within one or two years after delivery.

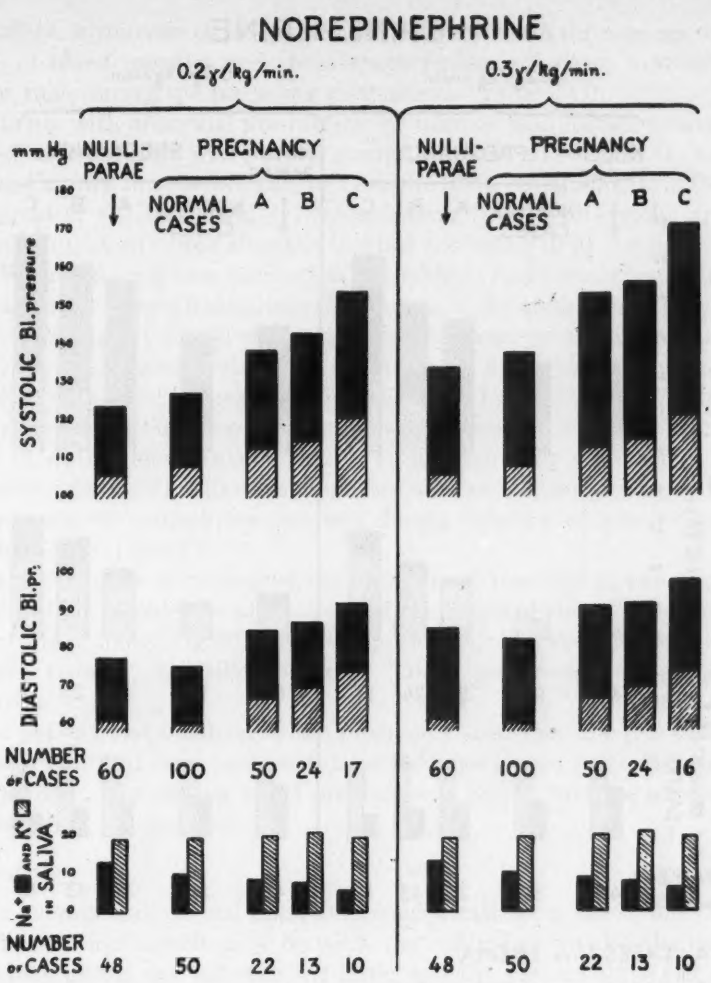

FIG. 1. Effects of norepinephrine infusion on blood pressure and electrolytes. The upper limits of the cross-hatched areas in the two upper rows of columns represent the average resting blood-pressure levels of the respective groups of subjects. The black areas in these columns represent the absolute blood-pressure elevations resulting from the infusions of norepinephrine. The bottom row shows the average salivary sodium (black) and potassium (cross-hatched) concentrations in the respective groups.

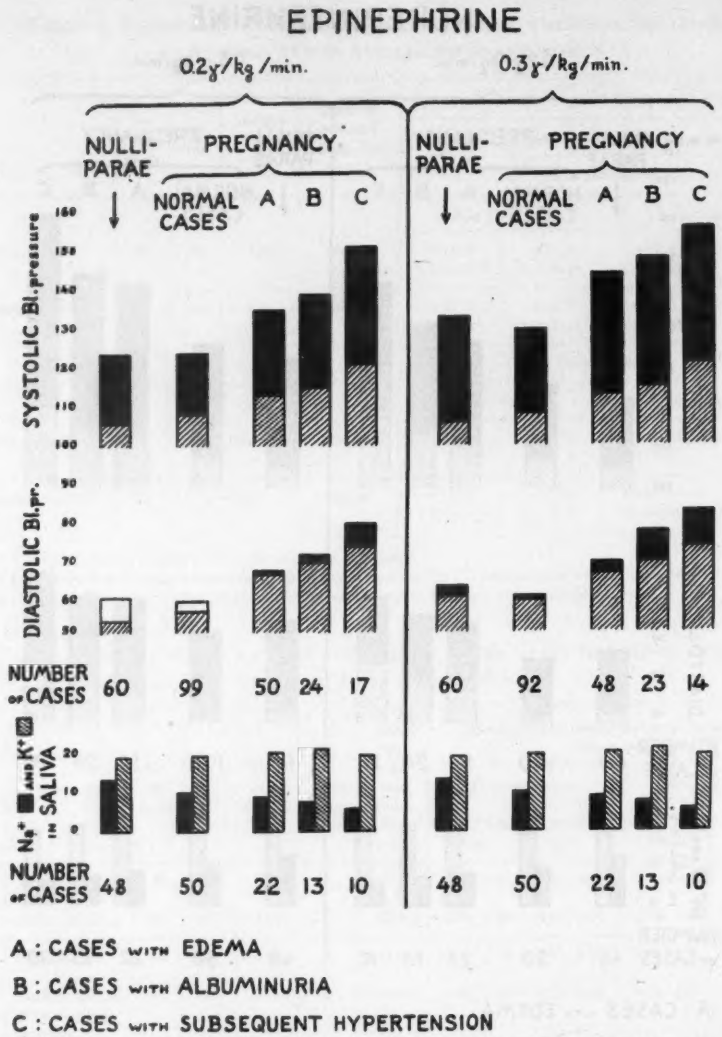

FIG. 2. Effects of epinephrine infusion on blood pressure and electrolytes. The upper limits of the cross-hatched areas in the two upper rows of columns represent the average resting blood-pressure levels of the respective groups of subjects. The black areas in these columns represent the absolute blood-pressure elevations resulting from the infusions of epinephrine. In the first two diastolic-pressure columns, an epinephrine-induced fall of the diastolic pressure is indicated by a white area, the top of which corresponds to the resting blood-pressure level. The bottom row shows average salivary sodium (black) and potassium (cross-hatched) concentrations in the respective groups.

nant state, within one to two years after delivery. Both the average resting levels of blood pressure and the average pressor responses were slightly higher than during the preceding pregnancies (Table 2 C).

Patients with abnormal pre-toxemic or toxemic manifestations were arranged in 3 partially overlapping groups, namely, those with (a) edema (noticed at any time before and/or after the test), (b) albuminuria (before and/or after the test), and (c) hypertension (from 140 systolic and 90 diastolic up) at any time after the test but not before or at the time of the test. When the tests were carried out, all subjects had normal resting blood pressure levels. The edema group (50 cases) and the albuminuria group (24 cases) had slightly elevated average resting blood pressures and significantly increased pressor responses as compared with the 100 cases of uncomplicated normal pregnancy (Table 3; Figs. 1 and 2). The 17 patients with subsequently developing hypertension presented the highest normal range of resting levels (average 121/74) and markedly increased systolic pressor responses. The diastolic responses were significantly increased during infusion of epinephrine but not during infusion of norepinephrine (Table 3; Figs. 1 and 2).

Eleven patients were singled out as cases of maximal hyper-reaction. Their systolic response to at least one of the catechol amine infusions was 61 mm. Hg or more (Fig. 3). As shown in Table 4, all of these patients were, or later became clinically abnormal. Three progressed to full-fledged eclampsia.

One patient, not included in the pregnancy statistics (the last in Table 4) was tested four days post partum, after having been pre-eclamptic before delivery. The resting blood pressure was 138/90 and the pressor responses were exaggerated.

Electrolytes

The average sodium and potassium concentrations in the serum (Table 5) did not differ significantly between the control group and the normal pregnancy group, nor between the latter and the various abnormal pregnancy groups. The average salivary potassium concentration (Table 5) was likewise nearly the same in all groups, but the average salivary sodium level varied considerably. The highest average value was observed in the non-pregnant nulliparous control group. A significantly lower sodium average was found in the normal pregnancy group. It declined further in the abnormal pregnancy groups in the following order: edema group, albuminuria group, pre-hypertensive group. Due to wide individual variations and the relatively small number of observations, these latter differences from the normal average pregnancy values were not significant at the 5 per cent probability level (Fisher's *t*-test). However, they gained

TABLE 3. BLOOD PRESSURE RESPONSES TO CATECHOL AMINES IN NORMAL, PRE-TOXEMIC AND TOXEMIC PREGNANCIES

	No. of cases	Age (avge.)	Weight (lbs.) (avge.)	Basal bl. pressure (mm. Hg) (avge.)	Blood pressure deviations from basal level (averages)			
					Systolic 0.2 μg./Kg. min.	Diastolic 0.3 μg./Kg./ min.	Systolic 0.2 μg./Kg./ min.	Diastolic 0.3 μg./Kg./ min.
					Norepinephrine			
Normal pregnancies	100	25	150	108/60	+19.5 (± 9.4)*	+15.7 (± 7.1)	+29.3 (±12.4)	+21.4 (± 9.5)
Cases with edema§	50	24	143	113/67	+26.6† (±15.2)	+18.8† (± 8.3)	+40.0‡ (±20.5)	+23.6 (± 9.5)
Cases with albuminuria‖	24	23	139	115/70	+28.5‡ (±18.5)	+17.4 (± 8.7)	+41.5‡ (±26.1)	+21.6 (±10.8)
Cases with hypertension¶	17	24	145	121/74	+33.2‡ (±22.4)	+18.3 (± 9.8)	+50.5‡ (±29.6)	+23.8 (±11.9)
					Epinephrine			
Normal pregnancies	100	25	150	108/60	+16.0 (± 8.9)	− 2.6 (± 8.8)	+22.5 (±10.8)	+ 0.6 (± 8.9)
Cases with edema§	50	24	143	113/67	+22.3‡ (±17.7)	+ 0.6† (±10.4)	+31.3‡ (±19.4)	+ 3.0 (±11.2)
Cases with albuminuria ‖	24	23	139	115/70	+24.3‡ (±18.0)	+ 1.8‡ (±8.4)	+33.5‡ (±22.4)	+ 8.0‡ (±10.6)
Cases with hypertension¶	17	24	145	121/74	+30.2‡ (±22.7)	+ 5.2‡ (±10.9)	+35.2‡ (±22.5)	+ 8.6‡ (±12.0)

* The figures in parentheses indicate the standard deviations.
† Difference from the normal pregnancy group significant at the 2–5 per cent probability level (*t*-test).
‡ Difference from the normal pregnancy group significant at the 1 per cent probability level (*t*-test)
§ In 22 of these cases the edema appeared after the test.
‖ In 10 of these cases the albuminuria appeared after the test.
¶ In all of these cases, hypertensive blood-pressure elevations (from 140 systolic and from 90 diastolic up) appeared after the test.

added significance by recent analogous observations of other investigators (55). The Na/K ratio behaved in conformity with the sodium concentrations, with the highest average (0.7) in the non-pregnant group and the lowest (0.3) in the pre-hypertensive group.

DISCUSSION

The observations reported here may be regarded from the dual point of view of their pathogenic implications and of their possible diagnostic and prognostic usefulness.

A. *Normal pregnancies*

The absence of any major difference between the nulliparous controls and the normal pregnant women regarding resting blood-pressure levels and

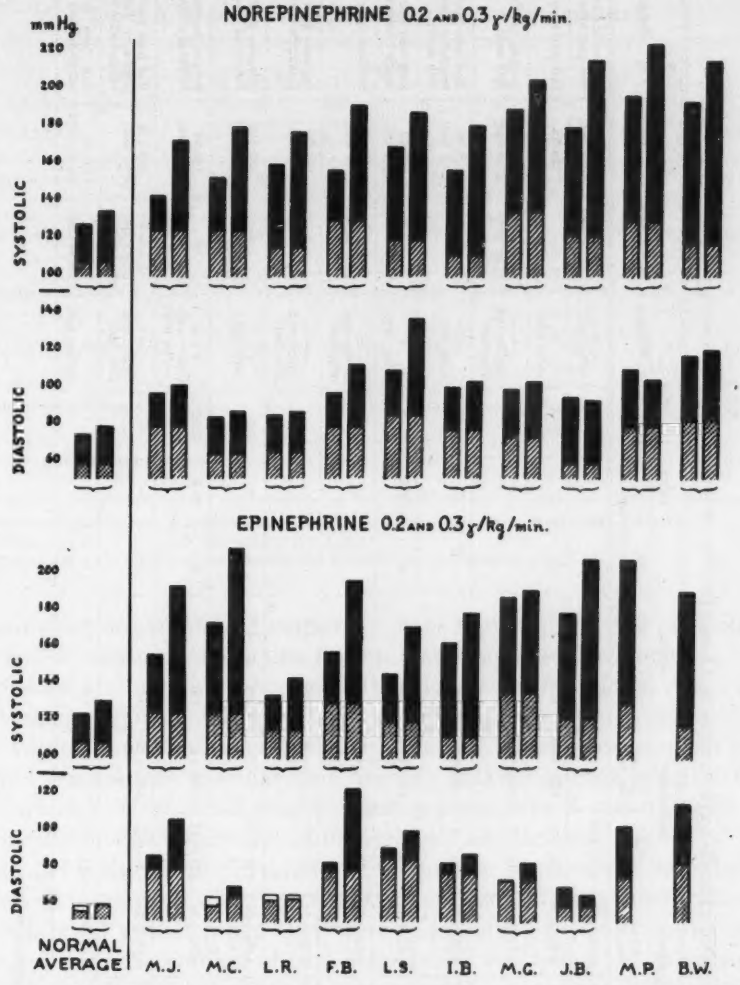

FIG. 3. Effects of infusion of catechol amines on blood pressure in 10 individual hyperreactors, compared with the averages in 100 normal pregnant women. The upper limits of the cross-hatched areas represent the resting blood-pressure levels; the black areas represent the absolute blood-pressure elevations during infusions of norepinephrine (two upper rows) and epinephrine (two lower rows). The averages for 100 cases of normal pregnancy appear on the left of the vertical line. In each pair of columns, the one to the left corresponds to the effect of 0.2 microgram/Kg./min., and the one to the right corresponds to the effect of 0.3 microgram/Kg./min. of the respective infused catechol amine.

TABLE 4. BLOOD PRESSURE AND ELECTROLYTE LEVELS IN 11 MAXIMALLY RESPONDING PREGNANCY CASES (1 POST PARTUM)

Patient	Age (yrs.)	Pregnancy number	Weeks of pregnancy	Weight (lbs.)	Basal blood pressure (mm.Hg)	Norepinephrine		Epinephrine		Serum		Saliva			Edema	Albuminuria	Highest recorded B.P.	Remarks
						0.2 μg./Kg./min.	0.3 μg./Kg./min.	0.2 μg./Kg./min.	0.3 μg./Kg./min.	Na^+ (mEq./L)	K^+ (mEq./L)	Na^+ (mEq./L)	K^+ (mEq./L)	$Na^+/\triangle^+$ ratio				
B.W.	21	1	34	154	118/84	+76/+34	+98/+37	+78/+32	—	137	3.8	—	—	—	+++ (after test)	−−	160/112 (after test)	Mother had been toxemic. Salt-poor after 34th week.
M.G.	35	7	33	161	135/74	+55/+26	+70/+30	+53/+1	+56/+10	138	3.8	9	21	0.43	+ (after test)	−−	158/95 (after test)	Salt-poor after 24th week.
L.R.	41	4	33	120	116/66	+44/+20	+61/+22	+18/−3	+28/−2	—	—	—	—	—	+++ (before and after test)	++ (after test)	146/96 (after test)	Serpasil and Diamox after 26th week.
M.C.	31	6	24	224	125/65	+28/+21	+55/+23	+49/−5	+89/+6	140	4.2	4.6	17	0.27	++ (after test)	−−	135/85 (after test)	Had been toxemic in fifth pregnancy. Salt-poor after 13th week.
F.B.	24	2	30	134	130/80	+27/+18	+62/+33	+28/+4	+67/+44	135	4.0	8	17.5	0.45	+ (after test)	+ (after test)	130/90 (after test)	Had been edematous during first pregnancy.
M.J.	22	2	33	178	125/80	+18/+18	+48/+22	+32/+7	+69/+27	—	—	—	—	—	+ (before and after test)	++ (after test)	125/85 (after test)	Salt-poor after 37th week.
J.B.	25	1	37	189	112/78	+45/+23	+69/+26	+51/+5	+67/+10	139	4.6	—	—	—	+++ (before and after test)	++ (after test)	130/70 (after test)	Salt-poor after 24th week. Eclampsia developed after test.
M.P.	31	3	31*	144	130/80	+67/+31	+95/+25	+79/+24	—	136	4.9	—	—	—	+++ (after test)	++ (before and after test)	170/130 (after test)	Salt-poor after 30th week. Eclampsia developed after test.
J.B.	19	1	31	184	122/60	+60/+36	+94/+34	+58/+11	+87/+6	140	—	9.1	28.6	0.32	+++ (before and after test)	+ (before and after test)	146/95 (after test)	Became toxemic again in following pregnancy.
L.S.	22	2	33	120	120/86	+49/+24	+68/+52	+26/+6	+52/+15	—	—	—	—	—	+ (before and after test)	++++ (before and after test)	190/110 (after test)	Had been toxemic in first pregnancy. Eclampsia developed after test.
D.P.	39	14	—†	206	138/90	+40/+18	+63/−1	+67/+32	—	142	4.2	—	—	—	+ (before test)	+ (before test)	142/92 (before test)	Had been pre-eclamptic before delivery.

* Had reacted normally in thirtieth week.
† Post partum, four days after delivery. Not included in pregnancy statistics.

TABLE 5. ELECTROLYTES IN SERUM AND SALIVA (AVERAGES)

Type of cases	No. of cases	Serum		No. of cases	Saliva		
		Na^+ (mEq./L)	K^+ (mEq./L)		Na^+ (mEq./L)	K^+ (mEq./L)	Na^+/K^+ ratio
Nulliparous controls	56	141	4.2	48	13.8* (±9.3)	19.7 (± 2.4)	0.70
Normal pregnancies	143	138	4.2	50	10.2 (±7.4)	1.98 (± 3.7)	0.52
Pregnancies with edema	42	139	4.1	22	8.8 (±5.8)	20.5 (± 3.9)	0.43
Pregnancies with albuminuria	20	138	4.2	13	8.1 (±5.0)	21.3 (±14.9)	0.38
Pregnancies with ultimate hypertension†	14	138	4.2	10	6.2 (±2.7)	20.2 (± 5.0)	0.30
Pregnancies with maximal pressor response	7	138	4.2	4	7.8	21.0	0.35

* Difference from the normal pregnancy group at the >2 per cent probability level (*t*-test, Fisher).
The figures in parentheses indicate standard deviations.
† At the time of the tests, these pre-hypertensive patients were still normotensive.

pressor reactions to infused catechol amines suggests that the physiologic increase of corticoid production during pregnancy (*see* Introduction) does not exert a profound influence on the cardiovascular functional state. Yet, the hemodynamic characteristics of normal pregnancy (increased circulatory volume and cardiac output (34)), as well as indications of an augmented sympathetic vascular tone (56, 57), make it appear probable that the normal level of blood pressure during pregnancy is maintained by a specifically modified interplay of homeostatic mechanisms.

Age and body weight did not affect the pressor responses to the catechol amines. It is not clear why these responses appeared slightly accentuated in a group of 33 women when they were re-examined several months after delivery, since the number of past pregnancies was found not to influence vascular reactivity appreciably. A larger number of statistical observations would be needed to decide whether or not vascular sensitivity in multiparous women is somewhat increased in between gestational periods, only to return to normal during subsequent pregnancies.

B. *Hormonal pathogenesis of toxemia*

As far as the pathogenesis of toxemia is concerned, our results lend strong support to the concept that an excessive production of sodium-retaining corticoids constitutes an important pathogenic factor in the origin of toxemic hypertension as well as of other toxemic manifestations (edema, al-

buminuria). This conclusion is based on the conspicuous analogies which we found in the behavior of both the salivary electrolytes (low Na/K ratio) and the vascular reactivity to the sympathogenic pressor catechol amines (intensified pressor responses) in DCA-treated normal males on the one hand and in pretoxemic or toxemic women on the other. Recent reports concerning an abnormally augmented urinary excretion of sodium-retaining corticoids before the onset of, and during the toxemia of pregnancy (*see* Introduction) lend added conclusiveness to the interpretation of our findings.

The depression of the salivary Na/K ratio during pregnancy appeared to be maximal in pre-toxemia and toxemia. This is in agreement with analogous observations in pregnancy and toxemia cases which have been recently reported by Soiva and Parviainen (55) after completion of our own data. The lowered salivary sodium concentration resembles the effects of both DCA (54, 58) and aldosterone administration (59, 60) and is, therefore, not exclusively specific for either one of these. The absence of a reduction of serum potassium concentration in our pre-toxemic and toxemic pregnancy cases differs from the typically low serum potassium levels seen under the influence of DCA (4, 46). This detail may possibly suggest aldosterone activity, since the latter, in contrast to its marked sodium-retaining power, has been found considerably less effective in promoting the excretion of potassium (61). Recent investigations have presented evidence of an increased aldosterone production in toxemia of pregnancy (43, 44).

Taking all in all, we arrive at the conclusion that toxemic hypertension is caused by an exaggerated formation of sodium-retaining corticoids, possibly aldosterone, and their effect on the intra-extracellular cationic gradient of the contractile vascular elements. These electrolyte changes would sensitize the vessels to the intrinsic sympathogenic and blood-borne pressor catechol amines and possibly also to other pressor agents. It is worthy of note that, in our experience (45), DCA augmented the pressor response to infused catechol amines already at a time when no significant elevation of the resting blood pressure level had yet resulted, and also that the resting blood pressure of normal human subjects proved rather refractory to DCA (62). Thus, a prolonged and intense action of mineralocorticoids seems to be required for the sensitization of the vascular system to become manifest, not only in response to infused, relatively large, extra amounts of catechol amines but also to the small quantities of norepinephrine which are constantly present in the vascular walls (63, 64). The resulting hypertension-producing mechanism seems to operate in toxemic hypertension as well as in Cushing's syndrome, in Schroeder's "endocrine hypertensive syndrome" (23) and in Conn's "primary aldosteronism" (24). In cases of pheochromo-

cytoma which present toxemia-like manifestations during gestation but are more or less symptom-free between pregnancies, a pathogenic combination of excess pressor catechol amines (comparable to our infusions) with sensitizing corticoids seems to exist (65).

That the overactivity of the sodium-retaining corticoids which seem to cause toxemic hypertension may also contribute to the development of edema and albuminuria, is suggested by the behavior of the salivary electrolytes in the respective groups of our series (Table 5, Figs. 1 and 2) and by the increase of renal vascular resistance (66), which likewise may be attributable to corticoid-induced sensitization of the renal vessels to constrictor catechol amines. In normal pregnancy, on the other hand, the effective renal plasma flow and glomerular filtration rate have been found increased (67). Thus, edema and albuminuria were observed during three successive pregnancies in an otherwise asymptomatic case of pheochromocytoma, and failed to reappear in a pregnancy which followed excision of the tumor (65).

C. *Diagnostic and prognostic implications of the vascular hyper-reactivity test*

As no sharp borderline can be drawn in the individual case between the normal pressor response and an abnormally exaggerated response to infused catechol amines, we singled out as definite hyper-reactors the 10 patients in whom at least one five-minute average pressure rise exceeded 60 mm. Hg (Table 4, Fig. 3). All of these women were, or subsequently became clinically abnormal. Four were completely asymptomatic at the time of the test; 8 had albuminuria, then or later; and 9 had edema, then or later. In 8, hypertensive resting levels of blood pressure appeared after the test, and in 3, full-fledged eclampsia developed.

Undoubtedly the number of recognized hyper-reactors would have been higher if some who presented a normal reaction, but showed pre-toxemic symptoms subsequently during pregnancy, had been re-examined at a later date. In only 1 case (the seventh in Table 4) did we have such an opportunity. Although the first test had yielded a normal result and no clinical signs had been detectable, a second test, made seven weeks later and in the presence of edema, elicited a definitely abnormal response. Albuminuria appeared subsequently and eclampsia developed before term. Seven of the 17 patients who were normotensive at the time of the test (twenty-seventh to the thirty-third week—average, thirtieth week) but in whom hypertensive blood pressure levels developed afterward, had shown normal pressor reactions.

The fact that a "salt-poor" diet had been prescribed by their obstetricians for many of the pregnant women of our series at various stages of pregnancy prior to our tests (Table 4) may have reduced the number of

hyper-reactors and the degree of hyper-reaction, as we have found (22) that dietary salt restriction diminishes or even abolishes both vascular responsiveness to the catechol amines and the sensitizing effect of DCA. Introduction of a strictly salt-poor diet after the test may have partially or completely prevented the appearance of toxemic manifestations. In the patients' interest, we felt obliged to recommend such a regimen for all those whose pressor reactions were considerably above average.

There were 4 entirely symptom-free hyper-reactors, and 3 others had only edema at the time of the test. Edema being a very common phenomenon, present in as many as two thirds of all "normal" pregnancy cases according to one report (68), impending toxemia had been unsuspected in these 7 patients until the infusion tests revealed their abnormal vascular disposition. Two of the 7 had been toxemic in previous pregnancies, 5 subsequently became hypertensive, and 1 became eclamptic. It is of interest that 1 patient (the first in Table 4), a young symptom-free primipara in whom marked edema and hypertension developed later, reported that all of her mother's pregnancies had been complicated by toxemia.

One patient (the last in Table 4) who had been toxemic during pregnancy was examined four days post partum; she still showed marked vascular hypersensitivity.

Despite the small number of definite hyper-reactors in our series, the close relationship between an excessive vascular hyper-reactivity on one hand, and the threat of impending toxemia on the other, seems clearly enough established to consider the catechol amine infusion test as a possible diagnostic or prognostic procedure, suitable for identifying among clinically asymptomatic pregnant women those who are approaching serious complications and need the immediate introduction of drastic prophylactic measures. For practical purposes, the following tentative recommendations are made at this time:

1. Asymptomatic pregnant women who have a history of parental or previous personal toxemia should undergo the norepinephrine test.

2. The test should also be performed in otherwise asymptomatic pregnant women, if they exhibit edema.

3. In general, it will probably be unnecessary to perform the test earlier than in the thirtieth week of gestation if the findings are normal. Repetitions at intervals of one or two weeks may be advisable.

4. It should suffice to limit each test to one five-minute infusion of *l*-norepinephrine (Levophed) in a dosage of 0.3 microgram per kilogram per minute; with this dosage there are hardly any side-effects aside from pallor and occasionally a slight headache during the infusion. (For technical instructions *see* section on Methods.)

5. If the average of the five systolic blood-pressure elevations above the

resting level should fall between 50 and 60 mm. Hg, the patient should be watched carefully as a probable candidate for toxemia; if the average is 60 mm. or more, the danger of impending toxemia appears acute, and the patient should be given a rigidly "salt-free" diet, containing not more than 300 mg. of sodium per day; additional prophylactic treatment with acetazolamide (Diamox), Rauwolfia drugs and 1-hydrazinophthalazine (Apresoline) may be considered. The latter has been found effective both in experimental (47) and clinical (69) toxemia.

It would be interesting, and might be useful to correlate results of the norepinephrine infusion test with those of Assali's "TEAC blood pressure floor test." When the latter reveals an abnormally weak depressor effect of tetraethylammonium chloride, it seems likewise to presage impending toxemia at a pre-clinical stage (70). Dieckmann's pituitrin test (71) is apparently based on the same principle of vascular hypersensitivity which we have demonstrated by means of catechol amine infusions, and might be equally informative. However, according to Dieckmann himself, it is fraught with dangers which do not need to be feared in either the norepinephrine or the TEAC test.

Determination of the level of serum electrolytes does not provide any useful information; that of the salivary electrolytes yields individual results which are too variable and influenced too much by interfering factors (such as time of the day and intake of meals) to be of practical diagnostic value (72).

SUMMARY

Standardized norepinephrine and epinephrine infusion tests in 60 nulliparous control cases and in 100 cases of completely normal pregnancy in the third trimester, did not reveal any significant influence of the state of pregnancy upon vascular pressor responsiveness. Neither did age, body weight and the number of past pregnancies affect the outcome of the tests.

Three partly overlapping groups of cases of abnormal pregnancy (50 with edema, 24 with albuminuria, and 17 with toxemic hypertension developing only after the tests) showed in that order a significant increase of average vascular pressor reactivity with these tests. In all of the 10 cases of maximal hyper-reaction, manifestations of toxemia were present or developed later.

Average sodium and potassium concentrations in the serum were practically identical in all groups. Average salivary potassium values were also nearly the same throughout, but average salivary sodium values and thus the salivary Na/K ratio, decreased in the following order of partly overlapping groups: non-pregnant controls, normal pregnancy, pregnancy with edema, pregnancy with albuminuria and pregnancy with toxemic prehypertension.

The vascular hyper-reactivity in toxemia represents an analogy to that which we had previously demonstrated in DCA-treated males, and the low salivary Na/K ratio in toxemia resembles that induced by both DCA and aldosterone. These analogies, correlated with the findings of other investigators regarding electrolyte balance and corticoid excretion, suggest that the syndrome of toxemia of pregnancy is caused by an overproduction of (placental ?) corticoids, among which aldosterone may occupy a prominent place.

The phenomenon of toxemic hypertension is interpreted as being caused by a corticoid-induced increase of the intra-extracellular cationic gradient, and by its potentiating effect on vascular cell contractility in response to the adreno-sympathogenic pressor catechol amines and possibly also to other, as yet unidentified, pressor agents. The catechol amines are believed to serve as direct stimulators of the contractile vascular cells, the mineralocorticoids as slowly-acting sensitizers, and the corticoid-regulated intracellular sodium ions as the obligatory mediators in the process of vascular sensitization. In these respects, the hypertension of toxemia may be considered as a revealing example for the understanding of the mechanism of other types of corticoid-induced and catechol amine-induced ("hormonal" and "neurogenic") arterial hypertension.

The norepinephrine hyper-reactivity test (excessive response to 0.3 microgram per kilogram per minute intravenously for five minutes) may be used, beginning with the thirtieth week of gestation, for the detection of still asymptomatic pre-toxemia, so that vigorous measures may be applied in time to prevent or mitigate toxemic complications.

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EFFECT OF BILATERAL ADRENALECTOMY ON METASTATIC CHORIOCARCINOMA FROM TERATOMA OF THE TESTIS

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TESTICULAR cancer becomes apparent most frequently in the middle and late twenties, that is, near the height of sexual potency. This might suggest an androgenic influence. McClelland (1) performed bilateral orchiectomy in a 26-year-old man with embryonal-cell carcinoma of a testis. The clinically evident metastases vanished after a month. This patient, however, had three courses of deep roentgen therapy before being seen by McClelland. Although a large epigastric mass was present at the time of orchiectomy and disappeared thereafter, the history does not indicate whether the orchiectomy was performed in the immediate post-radiation period. If it were, regression might have been due to the radiotherapy. Saleeby (2) reported disappearance of a large retroperitoneal mass from a testicular teratoma in a 50-year-old man after bilateral orchiectomy and after the delivery of 3500 r to the mass from several fields. Description of the testicular tumor fits seminoma, and radiotherapy may have accounted for the regression of the tumor. Schwartz and Mallis (3) noted that bilateral orchiectomy did not seem to delay death from the metastases of testicular teratoma. Our patient showed no improvement from bilateral orchiectomy. The abdominal and supraclavicular masses continued to grow and the patient deteriorated rapidly. Hooker and Pfeiffer (4) reported that prolonged treatment of certain strains of mice with estrogen resulted in cancer of Leydig's interstitial cells and that androgen retarded formation of this metastasizing neoplasm.

Practically nothing is known about the effects of bilateral adrenalectomy on metastases of testicular cancer. Huggins and Bergenstal (5) briefly remarked that they found no effect in a case of chorionic epithelioma.

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METHODS

Quantitation of the effect of a presumptive therapeutic agent can be obtained from the growth rate of discrete metastases to the lungs before and after therapy.

Calculating the growth rate of essentially symmetrical lesions requires only elementary mathematics. Growth represents cellular multiplication and, when uniform, can be expressed as doubling-time, or the time required for a mass to double in volume. If cell death, necrosis, and edema are not extensive, the doubling-time of the mass will approximate the average intermitotic interval of the component cells. Doubling is logarithmic, resulting in more dramatic increase when one billion cells divide to two billion, than when a single cell becomes two, even though the doubling-time is the same. Calculation of doubling-time requires knowledge of the change in volume and the time during which this change takes place. The volume of a sphere is a function of the cube of its diameter ($V = 1/6\pi d^3$). Clinically it is common to remark loosely that a nodule "doubled in size." This customarily implies a doubling in diameter but the increase in volume or mass is eightfold. An increase in volume of eight actually requires three doublings ($1\times2\rightarrow2$; $2\times2\rightarrow4$; $4\times2\rightarrow8$). The relationship between diameter, volume, and doubling-time is:

$$\frac{\log \frac{V_2}{V_1}}{t} = \frac{\log 2}{D.\,T.}$$

$$D.\,T. = \frac{\log 2 \times t}{\log \frac{V_2}{V_1}} = \frac{.301 \times t}{\log \frac{1/6\pi d_2^3}{1/6\pi d_1^3}} = \frac{.301 \times t}{\log \frac{d_2^3}{d_1^3}}$$

where:

D. T. = doubling-time.
V_1 = volume at the first observation.
V_2 = volume at the second observation.
d_1 = diameter at the first observation.
d_2 = diameter at the second observation.
t = interval of time between the two observations.

The estimation of doubling-time has been simplified by the use of graph paper designed for this purpose (Fig. 3).

Sources of error and the degree of reliability of such measurements as well as doubling-times in a variety of metastatic lesions, and the significance of such observations in therapy of cancer, are discussed at length by Collins, Loeffler and Tivey (6). Figures 1, 2 and 3 demonstrate the use of the special graph paper. In the present case this method was utilized to evaluate the effect of bilateral adrenalectomy on the growth rate in the lungs of metastases of choriocarcinoma from a testicular teratoma.

CASE REPORT

In July 1954, this 34-year-old white man (VAH 46257) coughed up clotted blood on two occasions. In September he began having discomfort in the right upper quadrant

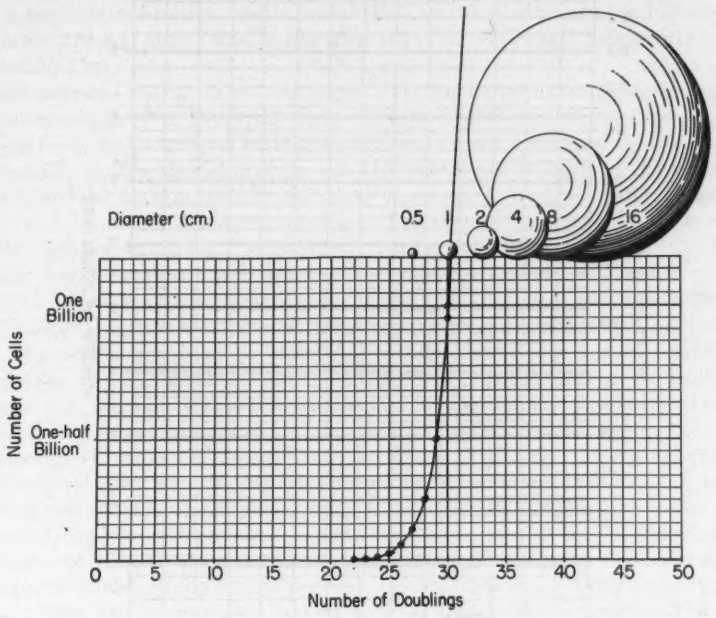

FIG. 1. Growth line of hypothetical tumor on arithmetic coordinates.

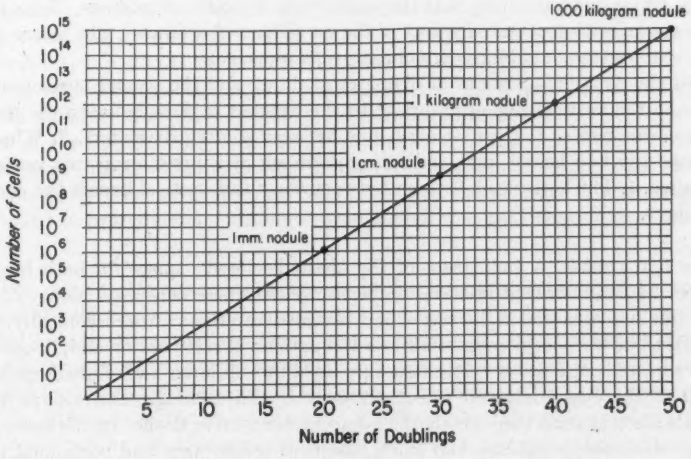

FIG. 2. Growth line of same hypothetical tumor on semi-logarithmic scale.

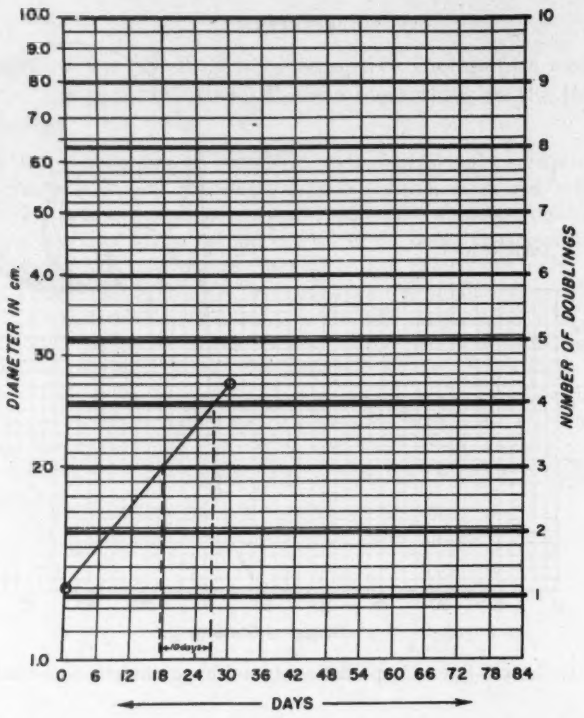

FIG. 3. Chart for estimating doubling-time from diameter of nodules. This graph is based on the logarithm of the cube root of the diameter (*left ordinate*). The heavy, evenly spaced lines represent doublings in volume (*right ordinate*).

1. Plot the first measurement of diameter at 0 day and the second measurement of diameter on the vertical line appropriate to the number of days between the first and second measurements. *2.* Draw straight line between plotted diameters. *3.* Where this line crosses any two heavy lines, indicating doubling of volume, drop two verticals to the base-line. *4.* The horizontal distance between these two vertical lines is the doubling-time in days.

where he felt a mass. A radiogram of the chest disclosed "spots" in both lungs. On September 24 he underwent bilateral orchiectomy elsewhere because a biopsy of an indurated but not enlarged right testis had revealed teratoma. Microscopically, in the right testis there were focal areas of fibrosis and patches of calcification and, in one region near the surface, there was a cystic structure lined by columnar and ciliated epithelium. The wall of the cyst contained smooth muscle, hyalin cartilage, and groups of well-formed glands scattered throughout the adjacent connective tissue. In the surrounding testis the seminiferous tubules had thick basement membranes and occasional tubules were completely hyalinized. Interstitial cells of Leydig were prominent. The left testis was normal. The diagnosis was adult teratoma[1] of the testis.

During September and October the patient continued to have increasing upper ab-

dominal pain unaffected by food and without nausea, hematemesis or melena. There was no gynecomastia or other female traits. His appetite became poor and his weight fell from 82 to 59 Kg. At this time he was admitted to the Veterans Administration Hospital, Houston, Texas.

His previous health record was good except for an operation for a ruptured intervertebral disc in 1953. There was no family history of cancer. At the time of admission he was fairly well developed but slightly undernourished. He appeared both acutely and chronically ill. The arterial pressure was 110 systolic and 70 diastolic. There was a hard mobile nodular mass in the left supraclavicular fossa, 3 cm. in diameter. A few fleeting râles were heard in both pulmonary bases. The abdomen was mildly tender, especially in the right upper quadrant where a large hard nodular mass was practically immobile during deep breathing. The spleen was not palpable. The edge of the liver was barely palpable below the right costal margin. It was smooth, round, and non-tender.

Radiographic studies disclosed no metastatic involvement of the skeleton. On September 29 a chest film revealed multiple large well-circumscribed metastatic lesions in both lungs (Fig. 4A). In another chest film, on October 28, the lesions (Fig. 4B) had increased in size and number. Intravenous pyelography indicated no significant abnormality other than medial displacement of the proximal portion of the right ureter. A right retroperitoneal mass was demonstrated by presacral pneumoretroperitoneography.

The blood count and the findings on urinalysis were normal. Results of an Aschheim-Zondek test on the urine were positive. A Kahn test of the blood yielded a negative reaction. The level of blood urea nitrogen was 16 mg. per 100 ml.; of serum acid phosphatase, 0.4 King-Armstrong unit; and of alkaline phosphatase, 5.9 Bodansky units. The prothrombic activity was 65 per cent; thymol-turbidity, 4 units; cephalin flocculation, 1 plus; and bromsulfalein retention, 7 per cent in thirty minutes. The levels of serum sodium, potassium and chlorides were within normal limits.

Pulmonary function studies revealed a vital capacity of 2,750 ml. (64 per cent of normal); tidal volume, 500 ml.; inspiratory reserve volume, 1,325 ml.; maximal breathing capacity, 68 liters per minute (56 per cent of normal); minute ventilation, 8.0 liters per minute; and a breathing reserve ratio,[2] 88 per cent (dyspnea-level is 70 per cent). The conclusion was that the patient had a moderate ventilatory defect.

Since testicular teratoma is rarely radiosensitive, the dose which could be delivered to both abdomen and thorax would not be expected to affect appreciably the natural course of the disease. The doubling-time for several of the pulmonary metastases was about twenty days, which indicated that survival would not exceed a few months. For this reason bilateral adrenalectomy was offered to the patient. It was pointed out to him that the procedure was entirely experimental and that there was a slender possi-

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[1] The primary testicular lesion known as "teratoma" not infrequently contains no malignant cells and may be termed adult teratoma. Friedman and Moore (7) have pointed out, however, that the term "benign teratoma" should never be used because metastases have occurred from testicular tumors which appeared to be adult teratomas. This is corroborated in the report of 100 cases of teratoma testis by Schwartz and Mallis (3). The 922 cases of testicular tumor of Friedman and Moore showed practically the same death rate and incidence of metastases for adult teratomas as for teratocarcinomas. These authors pointed out the trophoblastic potency of the teratoid tumors.

[2] $\frac{\text{Max. breathing cap.} - \text{Min. ventilation}}{\text{Max. breathing cap.}} \times 100$

FIG. 4. Radiographs of the chest taken at six feet. **A**, thirty-six days before adrenalectomy; **B**, seven days before adrenalectomy; **C**, twenty-two days after adrenalectomy. The spheroidal metastases, **a**, **b**, **c**, and **d**, are represented by the corresponding growth lines in Figure 5.

bility it would induce regression of the masses in his neck and abdomen. It was hoped that pain at least would be relieved.

On the day before adrenalectomy he was given 5 mg. of desoxycorticosterone acetate[3] (DCA) intramuscularly and 50 mg. of cortisone[4] intramuscularly in two places. On November 4 *bilateral adrenalectomy* was performed by the method of Hudson (8) under endotracheal anesthesia with ether and cyclopropane. During operation, hydrocortisone[5] (100 mg. in 500 ml. of 5 per cent dextrose) was administered intravenously at about 20 drops a minute. A large mass was seen adjacent to the right renal pedicle and right adrenal gland. The arterial pressure, which was normal and stable, did not fall after removal of the second adrenal gland and the patient never became cyanotic.

The left adrenal gland measured 6×2.5×1.5 cm. and weighed 7 Gm. The cut surfaces revealed a bright yellow cortex up to 0.1 cm. thick. The right adrenal gland measured 5×2×1.5 cm. and weighed 6 Gm. The cut surfaces were similar to those of the left adrenal. Microscopically, the zones in the cortex were discernible; the cells were pale and foamy. No change was noted in the medullary tissue.

A slow drip of hydrocortisone was maintained postoperatively until the amount administered was 100 mg. The arterial pressure remained uniform at about 120 systolic and 90 diastolic. The chest was clear bilaterally and the patient's color was normal. He complained little of pain at the incisions. On the first postoperative day he was given another 200 mg. of hydrocortisone by slow drip and sat up in a chair. On the second day the hydrocortisone was reduced to 50 mg. and he was ambulatory. On the third day he was exclusively on oral nourishment. Hormonal therapy now consisted of 20 mg. of hydrocortisone thrice daily and DCA, 2 mg. intramuscularly once daily. Deficits of sodium and chloride, which occurred during the first and second postoperative days, were corrected with 5 per cent sodium chloride given intravenously (*cf.* Maluf (9) for method of correcting such deficits). On the fourth postoperative day cortisone, 12.5 mg. every six hours, was substituted for the hydrocortisone because the former has a more potent positive effect on sodium and chloride reabsorption by the renal tubules and the patient tended to have a negative sodium and chloride balance. Negative sodium and chloride balance was also corrected by intravenous 5 per cent sodium chloride—the amount administered being indicated by the concentration in the serum and the patient's weight. On the eleventh postoperative day the enteric-coated sodium chloride was increased to 3 Gm. thrice daily.

Postoperatively the Aschheim-Zondek test on the urine was performed twice and the results were negative on both occasions. As the metastatic cancer grew, the patient's appetite deteriorated and he had to be aided with wine before meals, chlorpromazine (for nausea), and an occasional transfusion. On the twenty-second postoperative day, November 26, a chest radiogram showed massive infiltration with tumor (Fig. 4C). Nevertheless, the pain was considerably less than before the operation. During the final week, cortisone was given intramuscularly because the patient tolerated oral intake poorly. On December 15 his weight was 59 Kg. and the level of serum sodium was 130 mEq./L, potassium 5.2 mEq./L, and chlorides 86 mEq./L. On December 17, six weeks after adrenalectomy, the patient succumbed, apparently to severe loss of pulmonary reserve and to abdominal extension of tumor.

Measurements were made of four metastatic nodules from the roentgenograms taken on

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[3] Δ^4-pregnene-3,20-dione,21-acetate (deoxycortone).

[4] Δ^4-pregnene-17α,21-diol-3,11,20-trigone (compound E or cortone).

[5] Δ^4-pregnene-1β,17α,21-triol-3,20-dione (compound F or hydrocortone).

September 29, October 28, November 9, November 18, November 26, and December 7, 1954. The nodules measured were those marked a, b, c and d on the reproduction of the roentgenogram of October 28 (Fig. 4B). The nodules marked e, f and g were not included, even though they appeared to be clearly defined, because inspection of the film revealed that they were a conglomerate of several smaller nodules which were not quite discrete. The measurements were plotted on the special graph (Fig. 5). The increase in size of each nodule plotted best as a straight line over the entire period of observation. This

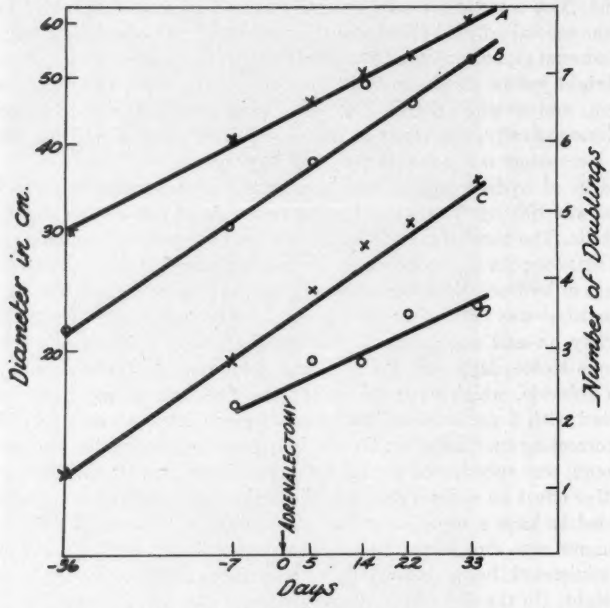


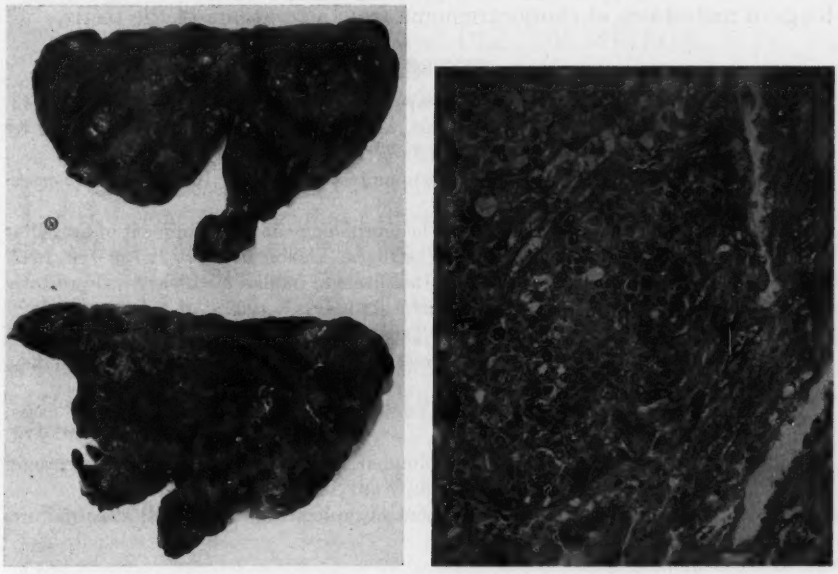
FIG. 5. Growth lines of specific metastases in the lungs of our patient (VAH 46257). *Left ordinate*: log plotting of cube of diameter. *Right ordinate*: doubling-time. *Abscissa*: time in days (minus sign indicates pre-adrenalectomy; plus sign indicates post-adrenalectomy) ***a***, ***b***, ***c***, ***d*** represent the spheroidal metastases shown in Figure 4B. Doubling times in days are as follows: for ***a***, twenty-three days; for ***b***, eighteen days; for ***c***, sixteen days; and for ***d***, twenty-four days.

indicated a constant growth rate for each, unaffected by adrenalectomy. It will be noted that the apparent doubling-times of the nodules were respectively 23, 18, 16 and 24 days. These were reasonably close and perhaps represented errors in measurement and technique rather than real differences in growth rate among the four nodules. The average doubling-time of 20 days may be compared with the range for tumors measured by Collins, Loeffler and Tivey (6), which extended from 11 to 164 days, and with their range for 8 testicular tumors with doubling-times of 11, 11, 13, 21, 22, 25, 30, and 153 days.

At necropsy the patient weighed 50 Kg. Both lungs were extensively replaced by neoplastic nodules up to 5 cm. in diameter, with little lung tissue remaining (Fig. 6A).

The left lung weighed 1,550 Gm., and the right lung weighed 1,775 Gm. In the retroperitoneal space, between the left lobe of the liver and the gallbladder, was a neoplastic mass (8×6 cm.) that displaced the duodenum forward. This tumor extended from the periaortic lymph nodes and surrounded but did not invade the right kidney and ureter. The pelvis of the right kidney was dilated. The liver weighed 1,800 Gm. and contained a few small neoplastic nodules. The prostate measured 3×2×2 cm. and appeared normal. Both testes were absent.

Microscopically, the neoplastic cells were arranged into varying sized nests and strands



A B

FIG. 6. Metastatic choriocarcinoma in the lungs. **A**. Gross specimen. **B**. Photomicrograph showing the lung pattern extensively replaced by varying sized, compact neoplastic cells, some with vesicular nuclei and abundant pale, stippled cytoplasm; others with small dark nuclei and little cytoplasm; and a few with multiple nuclei. (×200.)

surrounded by large fields of hemorrhage containing many endothelial-lined spaces (Fig. 6B). Some of the neoplastic cells were large, oval or angular, with vesicular nuclei and prominent nucleoli; others were elongated, with deeply stained nuclei; and still others were multinucleated.

COMMENT

Although it is plausible to consider that the growth of testicular tumors can be under hormonal control, there is no clear example of regression of a testicular tumor or of its metastases after orchiectomy or adrenalectomy

except in patients also receiving radiotherapy. In the patient of this report, who had chorionic metastases from a teratoma of the testis, bilateral adrenalectomy produced no apparent benefit. Serial radiographs of his chest showed no change in the growth rate of measured discrete metastatic nodules.

CONCLUSION

Bilateral adrenalectomy did not alter the constant growth rates in the lungs of metastases of choriocarcinoma from a teratoma of the testis.

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EFFECT OF INTRAVENOUS ADMINISTRATION OF TESTOSTERONE AND PROGESTERONE ON THE PLASMA 17-HYDROXYCORTICOSTEROID LEVEL IN MAN

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INTRODUCTION

DURING a study of the effect of testosterone on nitrogen balance (1), it became apparent that a drop in the plasma 17-hydroxycorticosteroid (17-OHCS) level regularly ensued following the administration of a large dose of testosterone (240 mg.) dissolved in human serum albumin. Subsequently, it became clear that this drop had two components, an immediate and profound effect associated with the testosterone infusion and a more gradual decline which was the result of the known diurnal variation of the plasma 17-OHCS levels (2). The studies which led to this conclusion are reported here.

MATERIALS AND METHODS

Ten male patients who were considered "normal" insofar as they had no obvious renal, hepatic or endocrine disease, were given a solution containing about 240 mg. of testosterone dissolved in 100 ml. of 25 per cent human serum albumin solution. This solution was administered intravenously over a period of about thirty minutes. The studies were always begun in the morning about 8 a.m. For each study, blood samples were drawn prior to the infusion and at 10, 30, 60, 120 and 360 minutes after the end of the infusion, which lasted from sixteen to thirty-five minutes. The plasma was separated within an hour and stored frozen, to be analyzed at a later date for 17-OHCS by the method of Nelson and Samuels (3).

On another day, each of these patients was given a solution of albumin without the testosterone and the studies were repeated.

Six of the subjects also received a solution containing 96 mg. of progesterone dissolved in 100 ml. of 25 per cent human serum albumin solution.

One subject received a solution containing 100 mg. of Δ5-pregnenolone.

In order to verify the apparent reduction in plasma 17-OHCS, 1 subject was given the usual testosterone infusion and, ten minutes after the completion of the infusion, a 200-ml. sample of blood was drawn so that 100 ml. of plasma was available for study by paper chromatography. A chromatogram of the extract of this sample was compared with that of a sample drawn the day before. By this means, one could ascertain whether

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or not the spot corresponding to cortisol was weaker after the infusion of testosterone. Each sample was extracted four times with 1.5 volumes of freshly redistilled chloroform. The extracts were evaporated to dryness in a water bath at 40° C. with a stream of nitrogen. The residue was dissolved in a small volume of methanol and chloroform (1:1 by volume), transferred to a strip of Whatman No. 2 filter paper and chromatographed in the benzene: 5 per cent aqueous methanol system described by Bush (4). As reference steroids, cortisone, cortisol, tetrahydrocortisone, tetrahydrocortisol, and compound S were chromatographed alongside the extracts.

The effect of the intravenous administration of testosterone solutions on the rise in the plasma 17-OHCS induced by administration of corticotropin (ACTH) was studied in 4 subjects. A six-hour intravenous infusion containing 25 mg. of corticotropin was given to each subject on two occasions and blood samples were drawn at two-hour intervals. On one occasion the testosterone solution as well as the corticotropin was administered to each patient. In 2 patients the testosterone solution was mixed with

TABLE 1. EFFECT OF TESTOSTERONE, PROGESTERONE AND ALBUMIN INFUSIONS ON PLASMA 17-OHCS LEVELS

MATERIAL	Patient	Control	Plasma 17-OHCS (γ/100 ml.)				
			Minutes after end of infusion				
			10	30	60	120	360
TESTOSTERONE	F.C.	15.5	2.5	2.6	3.7	1.5	16.7
	M.D.	8.5	0	1.8	3.5	3.5	6.7
	C.H.	22.5	4.9	5.4	6.6	6.5	11.2
	I.H.	16.3	0	0.3	1.2	—	11.9
	H.D.	18.7	7.5	6.7	7.4	—	4.8
	W.J.F.	11.0	0	1.2	3.1	5.6	7.5
	E.C.	8.7	—	0	0	1.7	8.5
	C.L.	18.8	2.3	1.1	8.0	12.5	6.1
	D.E.	9.6	2.9	3.6	2.4	8.1	6.9
	H.B.	21.0	0	0	4.0	10.0	11.0
	Mean	*15.1*	*2.2*	*2.3*	*4.0*	*6.2*	*9.1*
	S.D. of Mean ±	*1.6*	*0.9*	*0.7*	*0.8*	*1.4*	*1.1*
	*"P" value**	*0.9*	*<0.01*	*<0.01*	—	*>0.1*	*>0.1*
PROGESTERONE	F.C.	14.6	7.2	7.4	5.5		7.9
	M.D.	8.3	4.0	3.3	3.8	8.4	7.0
	C.H.	15.4	9.6	8.5	8.3	8.3	10.6
	I.H.	9.6	4.9	5.7	8.4	6.6	5.2
	H.D.	9.5	9.6	6.9	14.3	12.0	4.8
	W.J.F.	11.6	10.0	10.4	6.2	5.1	4.5
	Mean	*11.5*	*7.6*	*7.0*	*7.8*	*8.1*	*6.7*
	S.D. of mean ±	*1.0*	*1.1*	*1.0*	*1.5*	*1.2*	*1.0*
	*"P" value**	*>0.1*	*<0.02*	*>0.4*		*>0.7*	*0.9*

TABLE 1—(*continued*)

	F.C.	14.0	8.0			8.0
	M.D.	6.0		4.0	5.0	
	C.H.	20.0	16.0		9.8	9.4
	I.H.	14.2	10.0	7.5	6.4	1.7
	H.D.	13.1	15.0	15.2	6.0	1.7
	W.J.F.	15.8	11.5	10.7	5.9	6.0
	E.C.	11.6	9.2	8.7	4.3	9.8
	C.L.	24.8	21.2	13.4	13.1	5.4
ALBUMIN						
	H.D.	12.6	9.4	9.7	7.9	6.7
	W.J.F.	15.6	11.1	9.9	9.6	4.5
	E.C.	15.6	12.4	11.2	12.6	5.6
	C.L.	26.3	15.0	12.4	13.3	10.5
	D.E.	8.7	8.1	8.2	8.9	7.8
	H.B.	15.7	14.1	11.2	9.1	10.0
	Mean	*15.3*	*12.4*	*10.2*	*8.6*	*6.7*
	S.D. of mean ±	*1.5*	*1.1*	*1.0*	*0.9*	*0.8*
	"P" value†	*0.9*			*0.1*	*0.5*
	Diurnal variation	15.0		12.8	10.4	7.6

* The mean 17-OHCS levels in the plasma of subjects receiving testosterone and progesterone in human serum albumin solution were compared with those of subjects receiving only the albumin solution.

† The plasma 17-OHCS levels of the group receiving the albumin solution were compared with the expected diurnal variations in the plasma 17-OHCS of a group of subjects studied in our laboratory (5).

the corticotropin (Fig. 2, A and B), whereas in 2 patients the testosterone solution was administered only during the first half hour of the six-hour corticotropin infusion (Fig. 2, C and D).

Two subjects were given testosterone propionate in oil intramuscularly, 25 mg. daily over a ten-day period, to determine whether such therapy had any influence on the 8 a.m. levels of plasma 17-OHCS.

RESULTS

The plasma levels of 17-OHCS following the infusion of testosterone, progesterone and albumin are listed in Table 1. The mean values of 17-OHCS following the infusion are plotted in Figure 1. It may be seen that the administration of testosterone resulted in a lower level of 17-OHCS than one would expect from the administration of the albumin solution alone, and that this effect lasted more than one hour after the end of the infusion. By application of the *t* test, the mean values of 17-OHCS at ten and thirty minutes following the administration of testosterone were found

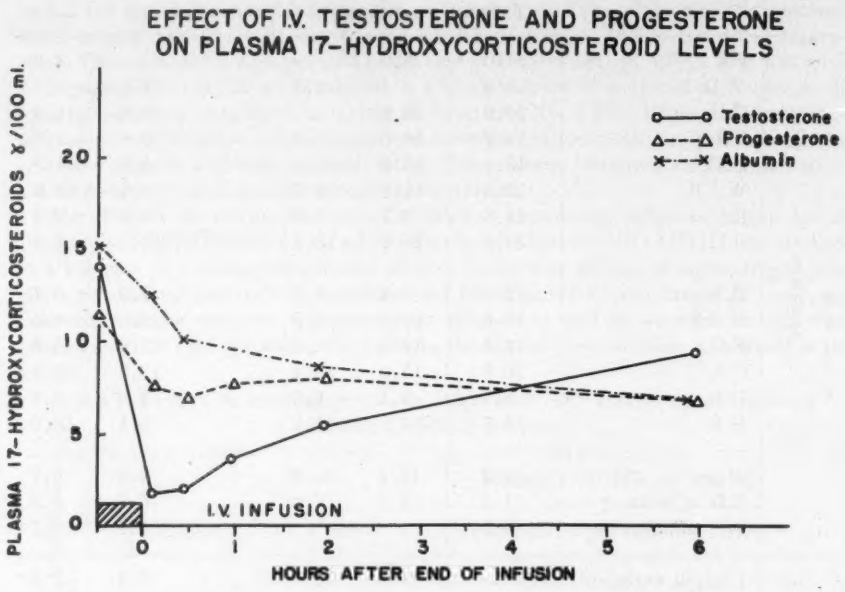

FIG. 1. The mean 17-OHCS levels of the plasma in group of subjects who received albumin, 240 mg. of testosterone and albumin, and 96 mg. of progesterone and albumin. The duration of the infusions was about thirty minutes. The apparent effect of progesterone is thought to be a sampling artifact as explained in the section on Results.

to be significantly different from the values of 17-OHCS in the plasma after the administration of albumin.

Progesterone infusions had little effect on the plasma 17-OHCS levels. The control and the two-hour post-injection levels of plasma 17-OHCS in the small series of patients receiving progesterone were lower than those of the group receiving albumin. This may well have been the result of drawing the initial blood samples of the progesterone series somewhat later in the day, inasmuch as the curve of the diurnal variation of the plasma 17-OHCS drops sharply at this time. The curve of the 17-OHCS levels after the albumin infusion was not significantly different from that of the spontaneous diurnal variation (2).

The infusion of pregnenolone had no effect on the plasma 17-OHCS levels in 1 patient.

There was no consistent effect of the testosterone solutions on the corticotropin-induced rise in the plasma 17-OHCS levels. This is illustrated in Figure 2, in which the results of four studies are plotted.

The administration of testosterone propionate in oil in a dose of 25 mg. per day intramuscularly for a period of ten days did not affect the morning

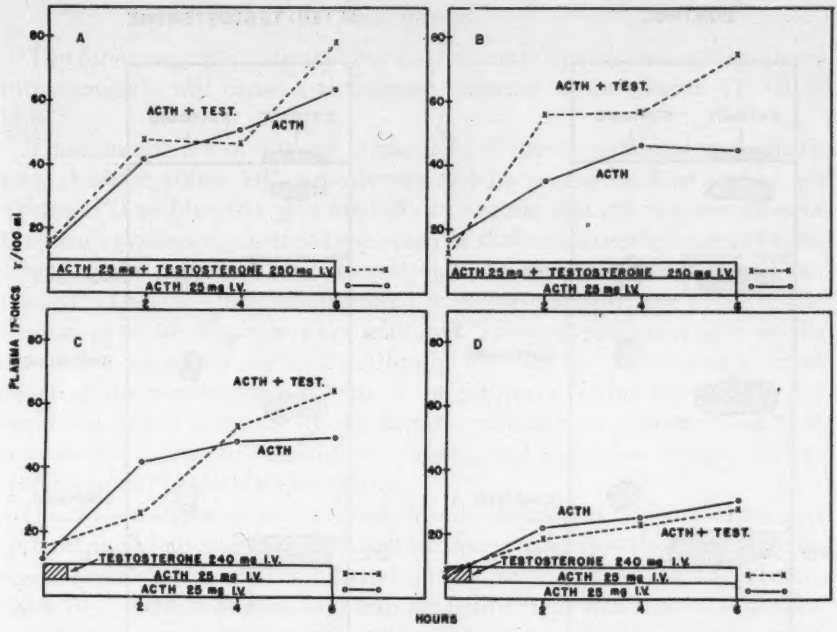

FIG. 2. The effect of a six-hour intravenous infusion of corticotropin (ACTH) on the plasma 17-OHCS levels in 4 subjects, with and without the simultaneous administration of testosterone. In studies A and B the testosterone was administered throughout the six-hour ACTH infusion, whereas in studies C and D the testosterone was administered only during the first half hour of the ACTH infusion.

level of 17-OHCS in the plasma. This amount of testosterone was enough, however, to put these 2 subjects in positive nitrogen balance.

Following chromatography, the spots absorbing ultraviolet light were delineated on paper with pencil (Fig. 3). The standards, cortisone, cortisol and compound S were visible. There was a dark spot with an R_f value of 0.06, a spot corresponding to cortisol ($R_f = 0.25$), a third diffuse light spot ($R_f = 0.55$) and a very dark band at the front of the chromatogram. The plasma extract after the infusion of testosterone did not show any spot corresponding to cortisol, and the first spot ($R_f = 0.06$) was lighter than that found on the chromatogram of the control plasma extract.

The area of the paper strips corresponding to cortisol was cut out, eluted with ethanol and the eluate was evaporated to dryness. The residue was dissolved in a known amount of methanol and two aliquots were taken. The Porter-Silber reaction (6) was run on one of the aliquots and the other was used as a blank. By this analysis, it was found that the control plasma con-

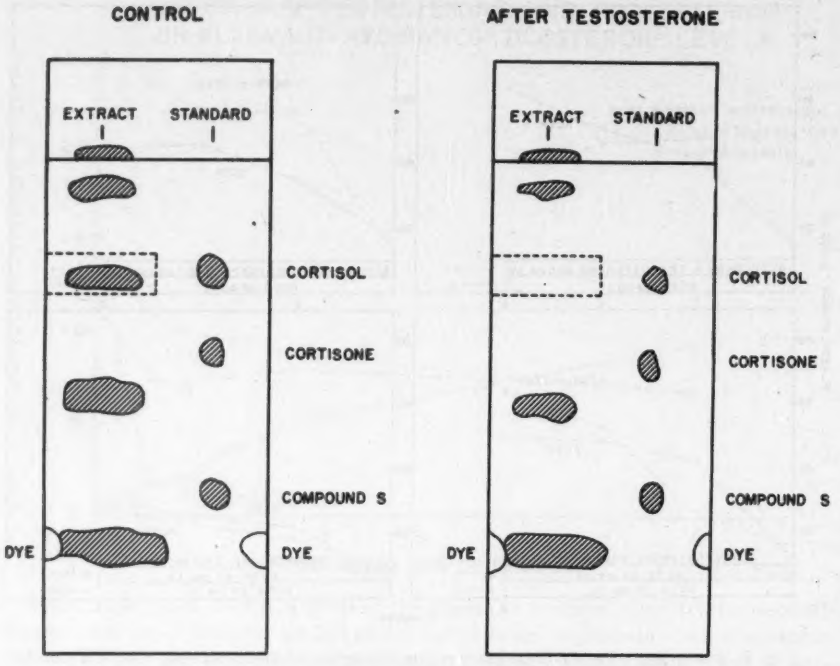

FIG. 3. Paper chromatograms of extracts of plasma from Subject C. D., before and ten minutes after the completion of the infusion of testosterone in albumin—as seen in the ultraviolet scanner. It is apparent that the spot corresponding to cortisol has disappeared from the chromatogram of the post-infusion plasma extract. The broken lines delineate the areas of the paper strip cut out and eluted for the Porter-Silber reaction.

tained 11.5 γ of cortisol per 100 ml. and the plasma obtained ten minutes after the testosterone infusion contained 2.5 γ of cortisol per 100 ml.

The remainder of the chromatograms was colored with blue tetrazolium. The standard compounds, including the tetra compounds, appeared as blue spots. No blue spot was found on either of the two chromatograms of the plasma extracts.

From this last experiment, therefore, it appears that the amount of cortisol in the plasma decreased from 11.5 γ per 100 ml. to 2.5 γ per 100 ml. following the intravenous infusion of testosterone, without the corresponding appearance of free reduced derivatives.

We were unable to demonstrate any consistent changes in the urinary corticoid excretion as measured by the modified technique of Reddy, Jenkins and Thorn (10) or by the technique of Glenn and Nelson (11) employing beta-glucuronidase.

DISCUSSION

The foregoing data indicate that testosterone, when given in large doses intravenously, will cause a temporary lowering of the plasma 17-OHCS level.

These results are of interest in the light of previous clinical and experimental observations with testosterone and its analogues. Bartter and coworkers (7) pointed out that methyltestosterone therapy in a few cases of Cushing's syndrome produced a marked anabolism of protoplasm and a decrease in the urinary 17-ketosteroid and 11-oxysteroid excretions. When methyltestosterone was administered with corticotropin, the effect of the exogenous corticotropin was not inhibited. These investigators came to the conclusion suggested earlier by Albright (8) that androgens had a direct effect on the corticotropin output of the pituitary. Zizine (9) came to the same conclusion from his studies in rats, in which he demonstrated that testosterone causes adrenocortical atrophy and a decrease in the corticotropin content of the pituitary gland.

Our observations on the effect of the administration of testosterone and corticotropin indicate that testosterone does not inhibit the effect of exogenous corticotropin on the adrenal output as measured by the plasma levels of 17-OHCS at two, four and six hours. This was true whether the testosterone was given over the entire six-hour period or whether a similar dose of testosterone was administered only during the first thirty minutes. Since the effect on the endogenous 17-OHCS levels was of relatively short duration, there may have been some effect during the first period. If such occurred, there was no significant persistence of effect from the second hour on.

The effect on endogenous plasma 17-OHCS levels appears to be specific for testosterone, since progesterone and pregnenolone caused no such change.

Some investigators have reported a drop in urinary 11-oxysteroid excretion (7) after continuous methyltestosterone administration. No consistent reduction was observed in this study; however, testosterone lowered the plasma 17-OHCS level for such a brief period that it would not be reasonable to expect this decrease to be observable in the 24-hour urinary excretion.

SUMMARY

1. The intravenous administration of testosterone in human serum albumin is associated with a marked but transient lowering of the plasma 17-OHCS level.

2. This effect was not noted when progesterone or pregnenolone was given in albumin solution.

3. The intravenous testosterone did not inhibit the corticotropin-induced rise in the plasma 17-OHCS levels.

Acknowledgment

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MYOTONIA ATROPHICA WITH TESTICULAR ATROPHY: URINARY EXCRETION OF INTERSTITIAL-CELL-STIMULATING (LUTEINIZING) HORMONE, ANDROGENS AND 17-KETOSTEROIDS*

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THE regular association of testicular atrophy and occasionally of other endocrine abnormalities with myotonia atrophica (dystrophia myotonica) has suggested that the disorder may represent a concatenated pluriglandular disturbance of endocrine origin and be susceptible to treatment by endocrine replacement therapy. An alternative interpretation is that myotonia atrophica, rather than reflecting a primary endocrine disturbance, is simply a manifestation of genetically linked degenerative phenomena seen in various tissues derived from embryonic ectoderm and mesoderm.

The disorder may be defined (1) as "a steadily progressive, familial, distal myopathy with associated weakness of the muscles of the face and levators of the eyelids, tendency to myotonic persistence of contraction in the affected parts, and testicular atrophy." In a search of the records of our hospital we found that testicular atrophy was present in all of the 7 patients with myotonia atrophica who were seen here. Atrophy of the testes was also recorded in 65 of 81 male cases of myotonia atrophica collected from the literature.

We have made detailed clinical, endocrine, and histologic studies of 2 typical cases (Figures 1–3). So far as we are aware, for the first time these studies have included histologic observations on testicular tissue correlated with measurements of urinary excretions of androgen, luteinizing (inter-

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stitial-cell-stimulating) hormone, follicle-stimulating hormone, and 17-ketosteroids.

DATA

Our endocrine findings (Table 1) agree with and extend those published by Jacobson, Schultz and Anderson (2) as well as the observations of

FIG. 1. *Case 1* (#95-054), aged 26. Myotonia. The patient had just clenched his fist and is opening his hands. The effect of delayed relaxation is evident.

FIG. 2. *Case 1*. External genitalia. Structures are normal except for testes, which measure 1×1×1.3 cm.

others, which are summarized in Table 2. No important abnormality was found in either of our 2 patients, other than those commonly recognized as manifestations of myotonia atrophica.

The age at onset of the disease in Case 1 was 10 years; in Case 2, 26 years. Muscular weakness and atrophy were local in Case 1, being confined chiefly to head, neck, forearms and hands, although myotonia was pronounced (Fig. 1). In Case 2, weakness and atrophy were generalized (Fig. 3) and tended to obscure the myotonia. As anticipated, the serum creatine level and urinary creatine excretion were elevated (Table 3). Frontal baldness was observed in each patient, and cataracts were found in Case 2.

FIG. 3. *Case 2* (#86-808), aged 53. Extensive atrophy and weakness.

In both men the testes were small and soft, the size of peas. Measurements (bilateral) were 1.0×1.0×1.3 cm. in Case 1, and 1.2×1.2×1.5 cm. in Case 2. The prostate and penis were of small normal size. Vasa and epididymides were small but well proportioned. Each man gave a history of having had occasional erections and ejaculations, but libido was difficult to evaluate. The mentality appeared to be normal. The findings on physical examination, except in respects noted, were normal. Blood pressure; pitch of voice; texture of skin; facial, body, axillary and pubic hair growth; subcutaneous fat distribution; breast development; and height-span ratio were all within the normal range for adult males.

There was no laboratory evidence of primary pituitary, adrenal, parathyroid or pancreatic disease. Roentgenograms showed the skull, chest, and bone age to be normal. The electrocardiogram, hemogram, hematocrit, blood smear, blood sedimentation rate, serologic reaction for syphilis, and results of urinalysis were normal for each patient. Chemical examinations of blood or serum yielded normal findings, including the following: fasting sugar, glucose tolerance, urea nitrogen, total protein, albumin-globulin ratio, cholesterol and cholesterol esters, calcium, phosphorus, sodium, potassium, chloride and carbon dioxide.

TABLE 1. URINARY 24-HOUR HORMONE EXCRETION IN 2 CASES OF MYOTONIA ATROPHICA

Urinary hormone	*Case 1* (man aged 26)	*Case 2* (man aged 53)	Normal range
Androgen, internat. units per day (3-day pool)	13.9±3.3	7.7±1.9	32–93
Luteinizing (interstitial-cell-stimulating) hormone, rat units/day	Greater than 24, less than 48	Greater than 12, less than 24	Less than 12
17-Ketosteroids, mg./day (3-day pool)	5.3	6.3	12–30
Follicle-stimulating hormone, mouse units/day	+5, 10; −50, 100, 200	+5, 10; −50, 100, 200	+5, 10, 20; −50, 100, 200

TABLE 2. SUMMARY OF REPORTED ENDOCRINE STUDIES IN MYOTONIA ATROPHICA

Author	Age of patient	Testicular atrophy	Testicular histology		Urinary 17-KS	Urinary FSH
			Interstitial cells	Tubules		
Jacobson *et al.* 8 cases (2)	28–61	In 6 of 8 cases	Normal	Atrophy common	Low normal	Normal in 4 cases studied
Sniffen (3)	40	Yes	Increased	Atrophic	Normal	Increased
Nadler *et al.* (4)	—	Yes	Normal	Atrophic	Low	Increased
Nadler *et al.* (4)	—		Slightly altered	Atrophic	Low	Normal
Zierler and Lilienthal (5)	58	Not stated	—	—	Low	Normal
Martin and Pattee (6)	55	Yes	—	—	Low normal	Normal
Benda and Bixby (7)	31	Yes	Degenerated	Hyalinized	Low normal	
Benda and Bixby (7)	44	Yes	Degenerated	Hyalinized	Normal	
Caughey and Brown (8)	45	Yes	Rare, vestigial	Atrophic	Low	Increased
Caughey and Brown (8)	28	Yes	Infrequent, degenerated	Atrophic	Low	Increased
DeCourt *et al.* 5 cases (9)	—	Yes			Low	

TABLE 3. CREATINE AND CREATININE METABOLISM IN 2 CASES OF MYOTONIA ATROPHICA

	Case 1	*Case 2*	Normal range
Serum creatinine (mg./100 ml.)	1.0	0.3	0–1
Serum creatine (mg./100 ml.)	0.5	2.2	Rarely found
Urinary creatinine (Gm./24 hrs.)	1.3	0.078	1.3– 1.5
Urinary creatine (mg./24 hrs.)	260.0	220.0	0–60.0

In neither patient was the thyroid gland palpable, nor was there clinical evidence of disturbed thyroid function. Abnormalities in laboratory data pertaining to thyroid function (Table 4) can probably be explained as a manifestation of the disturbed muscular development and function. The elevations of serum levels of protein-bound and total iodine noted in Case 2 were not due to recent ingestion of organic iodinated material.

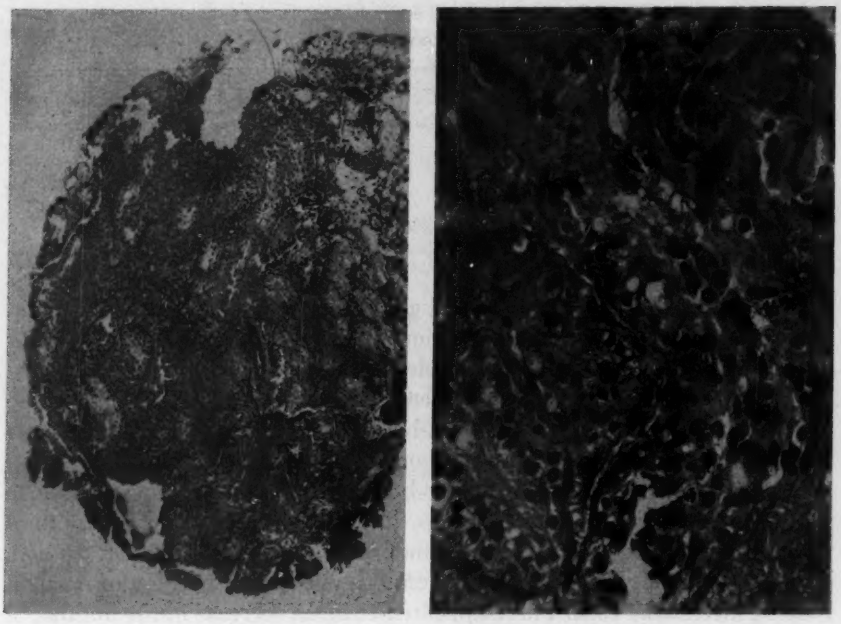
Of chief interest were the urinary hormone excretion studies (Table 1) and the testis biopsies (Figs. 4 and 5). The latter were carried out by Simmons' modification of Charny's technic. In the portions of tissue studied, there was atrophy of the spermatogenic tubules and clumping of normal-looking interstitial cells. There appeared to be an increase in the number of interstitial cells. Whether this was absolute or merely relative to the reduced aggregate mass of the testes is uncertain (10). Thickening of the tubular basement membrane, as observed in sclerosing tubular degeneration (Klinefelter's syndrome), was not found in our material.

Despite the apparently normal morphology of the interstitial cells, which like the adrenal cortex are supposed to be a source of androgenic hormones, the output of androgens in pooled urine specimens was greatly reduced, as measured by the comb-weight response of the immature chick (11). The daily 17-ketosteroid excretion in pooled urine was low (12).

Of considerable interest was the observation that the urinary excretion of interstitial-cell-stimulating hormone was greatly in excess of normal,

TABLE 4. THYROID GLAND FUNCTION IN 2 CASES OF MYOTONIA ATROPHICA

	Case 1	*Case 2*	Normal range
Basal metabolic rate (%)	−18, −20	−31, −15	
Radioactive iodine uptake (% dose in 24 hrs.)	39.9	1.27	20–50
Serum protein-bound iodine (μg./100 ml.)	5.4	12	4–8
Serum total iodine (μg./100 ml.)	8	13.6	5–9



A B

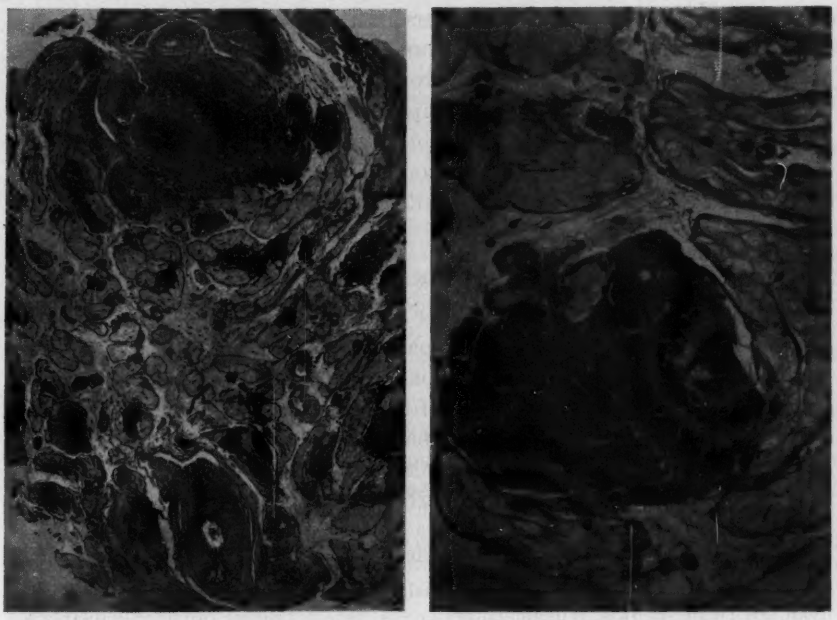
FIG. 4. Low-power (A) and high-power (B) views of histologic sections of testis biopsy specimen (Case 1). Clumps of normal interstitial cells adjoin the tubules, some of which are atrophic and some of which show severe spermatogenic arrest.

as measured by the increase in weight of the ventral prostate of the hypophysectomized male rat (13).

Albright and his associates suggested that the Sertoli cells may produce a hormone ("X hormone"), the analogue of estrogen in the female, which inhibits the interstitial-cell-stimulating (luteinizing) hormone of the pituitary. This hypothesis correlates well with the high interstitial-cell-stimulating hormone excretion and severe tubular atrophy seen in our 2 patients, in whom the Sertoli cells might be regarded as virtually inactive. It does not, however, explain the low urinary androgen output.

To complete the study of the pituitary-gonadal axis by available hormone assay methods, the urinary output of follicle-stimulating hormone was assayed, using a test based on the weight increase of the mouse uterus (14). Values were normal in each case.

The significance of these observations is not altogether clear. However, they show an association of abnormally high urinary excretion of interstitial-cell-stimulating hormone and low excretion of androgenic hormone



A B

FIG. 5. Low-power (A) and high-power (B) views of histologic sections of testis biopsy specimens (Case 2). Clumps of normal-looking interstitial cells; atrophic tubules.

in association with morphologically normal interstitial cells and atrophy of the testicular tubules in 2 cases of myotonia atrophica.

DISCUSSION

Testicular morphology. We have reviewed the literature and find that a histologic picture in the testis similar to that seen in our patients may occur in atrophy after mumps or in the adult cryptorchid testis (15–19). As far as we can tell, the histologic pattern also resembles that of Engle's "germinal aspasia" (19) seen in certain cases of infertility, and is analogous to findings which have been described in leprosy (20).

In the testicular atrophy associated with primary pituitary deficiency, there is atrophy of the interstitial cells as well as of the tubules (21).

The histologic findings in our patients are not strictly comparable with those in sclerosing tubular degeneration (Klinefelter's syndrome) (15, 22–25). Klinefelter's syndrome may generally be regarded as a primary tubular sclerosis with a compensatory increase in follicle-stimulating hormone. Thickening of the tubular basement membrane is a common if not

constant histologic finding. The interstitial cells are usually clumped and often do not appear normal. Gynecomastia and other eunuchoid features are not unusual. Urinary 17-ketosteroid excretion is normal or reduced.

Endocrine replacement therapy in myotonia dystrophica. There are reports of attempts to treat myotonia atrophica with testosterone propionate (26, 27), corticotropin (28, 29), and cortisone (11, 30, 31). It is difficult to evaluate the authors' conclusions, but results appear to have been poor or equivocal in the reports cited.

SUMMARY

Testicular atrophy occurs in 80 per cent of cases of myotonia atrophica. Detailed studies are reported on 2 patients in whom biopsy of the testes showed normal or increased interstitial cells in association with atrophy of the spermatogenic tubules. Assays of the urine showed follicle-stimulating hormone to be normal, 17-ketosteroids low, luteinizing (interstitial-cell-stimulating) hormone greatly increased, and androgens greatly reduced. No other evidence of endocrine disturbance was observed.

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POST-THYROIDECTOMY HYPOTHYROIDISM, HYPOPARATHYROIDISM, EXOPHTHALMOS AND GALACTORRHEA WITH NORMAL MENSTRUATION: METABOLIC RESPONSE TO PROBENECID

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THE following case is described because of persistent lactation in unusual circumstances and because of the unexpected metabolic response to probenecid (Benemid).

CASE HISTORY

H.W. (No. 116261) was a married white woman, aged 37 in 1955. In 1946, six months after the birth of her second child, her eyes became prominent, she tired easily and lost weight. A subtotal *thyroidectomy* was performed for what would appear to have been minimal thyrotoxicosis. (There was no increase in sweating, nervousness or bowel action.) The histologic picture of the removed thyroid tissue seems to have given rise to difficulty; it suggested toxic hyperplasia, but in some parts resembled Hashimoto's struma.

The postoperative course was marked by the occurrence of several complications: 1) *Tetany* supervened within three days, and has remained (although relieved by calcium lactate and calciferol). 2) *Myxedema* appeared within three months, and has been controlled by the administration of 4 grains of desiccated thyroid daily. 3) *Exophthalmos and ophthalmoplegia* progressed until 1948, in which year Sir Russell Brain was consulted. Since then the condition has remained stationary.

After the birth of the second baby in 1946, *lactation* persisted daily until 1954. For the first few years milk actually squirted out, but it slowly diminished until only a few drops could be expressed. It failed to respond to 5 mg. of stilbestrol daily or to testosterone propionate. In 1952 the patient became pregnant, but the lactation continued unabated. The pregnancy was terminated at $2\frac{1}{2}$ months. *Menses* remained perfectly normal, with a rather heavy flow lasting five days in regular monthly cycles.

Examination in 1954 revealed a well built, apparently euthyroid woman, neither obese nor hairy, with bilateral proptosis and lid retraction, paralysis of the right upward movement of the eyeball and wandering gaze. The eyeballs were not excessively tense and there was neither edema nor chemosis. The exophthalmos was not "malignant" at this time, in the sense that it was stationary. Chvostek's sign was present (calcium lactate only was being taken for the tetany, in a dosage of twenty 5-grain tablets daily). Gynecologic examination revealed no abnormality.

Investigations: (For methods, *see* Appendix.) The blood count was normal; Hb. 13.5 Gm. per cent. The Wassermann reaction was negative. The serum level of inorganic phos-

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phorus was 4.2–4.6 mg. per 100 ml.; calcium, 6.2–8.6 mg. per 100 ml.; alkaline phosphatase, 4.7 units (Shinowara); albumin 4.6 Gm.; and globulin 1.7 Gm. per 100 ml. The 24-hour urinary excretion of phosphorus was 600–1,100 mg. (intake about 1,000 mg.); of calcium, 105–208 mg. (intake about 800 mg.); and of 17-ketosteroids 5.4 mg. The titer of urinary FSH was positive at 6 mouse units at midcycle, and negative at 24 units. On x-ray examination, the skeleton, including the skull was normal. Endometrial biopsy showed a normal premenstrual endometrium.

The response to parathyroid hormone was a threefold rise in urinary phosphorus. The response to probenecid consisted of a decrease in the level of serum phosphorus and an increase in urinary calcium.

DISCUSSION

Calcium and phosphorus metabolism: vitamin D and probenecid

It was noticeable that the urinary calcium remained within the high-normal range even when the serum calcium level was only around 7.0 mg. per 100 ml., regardless of whether calcium lactate was being taken by mouth or not. Although it is usually stated that calcium virtually disappears from the urine when the serum level falls below 8 mg. per 100 ml., it is not uncommon to find cases of hypoparathyroidism with normal urinary calcium excretion but low serum levels. This suggests that the urinary calcium output is not governed solely by the serum level, but also by another mechanism, perhaps modifying the tubular reabsorption of calcium. In Patient H. W., 50,000 units of calciferol daily for ten days caused no change in the already normal daily output of urinary calcium, whereas the serum calcium rose from 7.5 to 9.6 mg. per 100 ml., and the Chvostek sign disappeared.

Probenecid has been claimed to increase the urinary excretion of phosphorus and lower the serum phosphorus level in hypoparathyroidism, but not in normal controls (1, 2). The administration of 1 gram of probenecid every six hours to H.W. induced no change in urinary phosphorus but lowered the serum phosphorus level from 4.7 to 3.7 mg. per 100 ml. (Fig. 1). The urinary calcium seemed to increase, the serum calcium concentration was not altered, and there was no change in the Chvostek or Trousseau signs. On repetition, the experiment yielded the same results (Table 1). The diet was constant during these periods. It is interesting that the response to probenecid was the same as that of a "pseudohypoparathyroid" patient with steatorrhea (3), and different from what we have found in normal controls. In both H.W. and the "pseudohypoparathyroid" subject also, probenecid was calciuric and caused a fall in the level of serum phosphorus without a corresponding phosphorus diuresis.

Galactorrhea

Lactation is usually associated with amenorrhea, as during normal suckling, in acromegaly, in the Chiari-Frommel syndrome of atrophy of

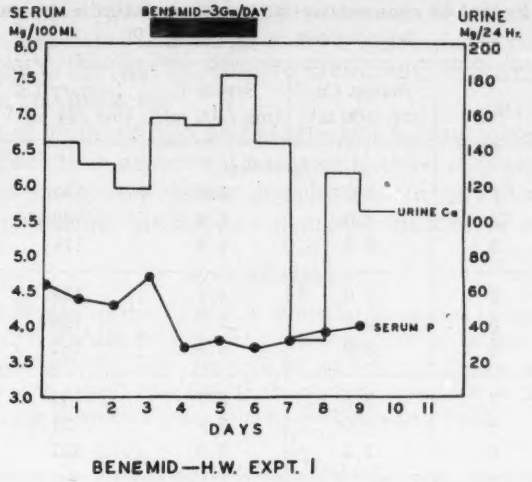

FIG. 1. Response of H.W. to probenecid (Benemid). Note rise in urinary calcium and fall in the level of serum phosphorus. The serum calcium level and urinary phosphorus were not altered, and are not charted.

uterus and ovaries with persistent lactation (4), and in the "lactation syndrome with low FSH" described by Forbes *et al.* (5) and by Argonz and del Castillo (6). In these cases, the estrogen production is low. In some instances pituitary FSH may be normal, as in lactating mothers. Now, as Forbes and her co-authors point out, a lack of estrogen cannot be essential for continued lactation, since some nursing mothers have regular periods and even become pregnant, and in some cases of the Chiari-Frommel syndrome, menstruation persists for a variable period (7). Furthermore treatment with estrogen does not usually check the abnormal galactorrhea (5, 8). H.W. similarly failed to respond to estrogen. Forbes *et al.* and Argonz and del Castillo suggest that in their patients the pituitary is specifically overproducing the hormone prolactin, believed to be formed by the eosinophil cells.

In the case of H.W. it is reasonable to assume that the tendency to hypothyroidism might stimulate the pituitary—at any rate with respect to thyrotropic hormone; however, this effect should be adequately counteracted by the administered thyroid. The persistence of the exophthalmos might also indicate an overactivity of the pituitary, perhaps in respect to the production of a special exophthalmos-producing hormone (9).

Zondek *et al.* (10) reported 5 somewhat similar cases which they believe to be caused by pituitary overactivity. These patients showed hyperthyroidism with exophthalmos, galactorrhea, and normal FSH. Unlike H.W.,

TABLE 1. EFFECT OF PROBENECID (BENEMID) ON CALCIUM AND PHOSPHORUS METABOLISM IN PATIENT H.W.

	Day	Serum Ca (mg./100 ml.)	Serum P (mg./100 ml.)	Urinary Ca (mg./24 hrs.)	Urinary P (mg./24 hrs.)
		Experiment I			
	1	8.0	4.6	144	864
	2	7.0	4.4	132	1,120
	3	8.5	4.3	118	797
	4	8.0	4.7	158	1,021
BENEMID	5	9.1	3.7	154	700
	6	8.0	3.8	182	689
	7	7.8	3.7	144	746
	8	8.0	3.8	31.5	327
	9	7.5	3.9	127	765
		Experiment II			
	20	8.6	4.6	—	486
	21	8.0	4.3	—	833
	22	7.8	4.5	—	—
	23	7.8	4.8	113	775
	24	8.0	4.6	92	826
	25	8.0	—	81	944
	26	8.0	3.8	120	875
BENEMID	27	8.0	3.7	123	820
	28	8.3	3.4	109	728
	29	8.2	—	128	902
	30	8.3	3.2	97	693
	31	8.6	—	104	630
	32	—	—	73	—

they were anemic, sterile, and had excessive uterine bleeding of the metropathic type with excessive estrogen production.

Salus (11) described a patient with hypothyroidism, galactorrhea and amenorrhea accompanying a tumor in the "pituitary duct." There is thus good evidence that pituitary overactivity enters into the picture in most cases of galactorrhea, and that some of these patients may have dysthyroidism without acromegaly. These points seem to apply to our patient, H.W. Certainly there can have been no lack of estrin in her case.

SUMMARY

The case of a woman suffering from hypoparathyroidism following thyroidectomy for doubtful thyrotoxicosis is reported. She also had a static

exophthalmic ophthalmoplegia and persistent lactation with normal menses. It is postulated that the pituitary is implicated in some way, showing a tendency to turn out an excess of thyrotropin, exophthalmos-producing hormone, and prolactin.

The effect of probenecid on phosphorus and calcium metabolism was investigated. Each time probenecid was administered it caused a fall in the level of serum phosphorus without any change in urinary phosphorus, and a rise in urinary calcium without any change in the level of serum calcium.

Acknowledgments

My grateful thanks are due to Prof. J. Brock for permission to observe his patient, H. W.; to Prof. F. Forman for constant interest and advice; to Prof. H. Zwarenstein and his staff (especially Mr. D. G. Duncan) for the FSH tests; to our technicians, to the dietitians of Groote Schuur Hospital, to the photographer (Mr. B. Todt) and to the University of Cape Town for a grant from its Staff Research Fund.

Appendix—Methods

Sérum calcium: Greenblatt and Hartman (13).
Serum and urinary inorganic phosphate: King (14).
Urinary calcium: Fiske and Logan (15).
Urinary FSH: Bloomberg *et al.* (16).

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ELECTROLYTE AND STEROID METABOLISM IN A PREGNANT ADDISONIAN

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PREGNANCY in a patient with Addison's disease affords a unique opportunity to study the steroid metabolism of the adrenal gland and placenta. Interest in this problem was initiated in 1943 by the publication of observations on the first Addisonian to complete a pregnancy at the University of Minnesota Hospitals (1). A more extensive study of the first such patient to complete pregnancy during therapy with cortisone alone is presented below. These observations raise questions concerning the source of the increased urinary pregnanediol in normal pregnancy, and the validity of the concept of adrenocortical activity of the placenta.

CASE PRESENTATION[1]

Mrs. J. N. was a 32-year-old para 1-0-0-1 at the time of her initial obstetrical visit to the University of Minnesota Hospitals on February 24, 1953.

Her first pregnancy, which terminated in May 1944, was uneventful except for the presence of albumin in the urine throughout the last trimester. In the last month a blood pressure of 137/90 was noted on one occasion, but there was neither edema nor symptoms of toxemia. At term, the urine contained 5 grams of albumin per liter. An uneventful surgical induction of labor was carried out, and outlet forceps delivery of a live 6½-pound female infant accomplished. The puerperium was unremarkable, albuminuria gradually lessened and the mother and infant were discharged on the eleventh postpartum day. At the routine follow-up examination, the patient's blood pressure was 110/70 and the urine contained only a faint trace of albumin.

The patient's health was not good thereafter. Her weight fell. By the summer of 1945, anorexia, amenorrhea and bronzing of the skin had developed. In the early fall of 1945, she was admitted to a hospital in northern Minnesota, where a diagnosis of Addison's disease was made and therapy with salt tablets and "injections" begun. Shortly thereafter she moved to Minneapolis.

In early December 1945, severe weakness and vomiting began. On December 11 she was admitted to Minneapolis General Hospital, having been comatose for one and a half hours. She was dehydrated, in a state of profound collapse and weighed but 69 pounds. She responded well to emergency treatment, and upon discharge shortly there-

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[1] Edmund B. Flink, M.D., Associate Professor of Internal Medicine of the University of Minnesota, cared for Mrs. N. with respect to Addison's disease before, during and after her pregnancy. J. Carlisle Brown, M.D., Atlantic City, N.J., cared for Mrs. N. during the first pregnancy and provided the information concerning it.

after received maintenance doses of salt and desoxycorticosterone acetate (DCA). She was referred to the University of Minnesota Hospitals for consideration of DCA pellet implantation.

Mrs. N. was seen in the out-patient department of University Hospitals for the first time in February 1946. The diagnosis of Addison's disease was confirmed by clinical and laboratory study, and, after a brief period of observation, DCA pellets were implanted. These and subsequently implanted pellets were the mainstay of therapy for the next four years.

In December 1946, Mrs. N. was hospitalized for coma precipitated by a minor upper respiratory infection. In September 1947, there was a precautionary admission at the time of a dental procedure. In October 1947, another admission was occasioned by an episode of diarrhea, which had precipitated a moderately severe metabolic disturbance. In July 1948, still another precautionary admission for a dental procedure was completed uneventfully. In March 1949, in the course of a severe tonsillitis, the patient became semicomatose and was again hospitalized. She once again recovered rapidly. In the periods between these admissions, the patient continued to manifest the stigmata of Addison's disease and was seriously limited in her activity. Blood pressure remained low and frequent supplementation of the pellets with oral DCA was necessary.

On November 7, 1950, oral cortisone therapy was begun with a dosage of 10 mg. a day. There was a very prompt appearance of a sense of well being, increase in work capacity and tolerance for minor illnesses. The cortisone was supplemented with oral salt tablets and small doses of thyroid. During the first year of therapy, intramuscular injection was tried and was no more satisfactory than oral administration. A dose of 12.5 mg. of oral cortisone a day was finally decided upon. The patient's menstrual pattern returned fully to normal for the first time since 1944.

Mrs. N. had her last menstrual period prior to pregnancy on December 20, 1952. On initial examination on February 24, 1953, she weighed 117 pounds. There was a generalized increase in pigmentation. Body hair was scant and there was virtually no hair on the labia. Scars of pellet implantation were visible. The obstetric pelvis was normal and the parous cervix was softened and blue. The uterine fundus was enlarged consistent with a nine weeks' intrauterine gestation. X-ray studies for pulmonary tuberculosis yielded negative results, as did the serologic test for syphilis. The patient was found to be Rh negative without antibodies and none developed in the course of pregnancy.

The prenatal course was uneventful. Nausea of early pregnancy was helped by changing the time of cortisone ingestion to the evening. After the thirteenth week the patient gradually returned to morning administration. After the twenty-eighth week, thyroid medication was stopped. There was a definite increase in pubic and leg hair. Albuminuria appeared on July 30, at the thirty-second week. The blood pressure remained stable at about 120/80 throughout the pregnancy. At the thirty-sixth week some pedal edema appeared and the patient reduced her salt intake. At no time, however, was there a striking gain in weight, and the maximum weight of 136 pounds was reached at the thirty-eighth week. Examination of the eyegrounds disclosed no evidence of vascular spasm.

Mrs. N. was hospitalized at the thirty-fourth week for study. Results of this period of observation have already been reported (2).

Labor started at 3:00 P.M. on September 30, 1953, and at 4:30 P.M. when labor became more emphatic, she took 50 mg. of cortisone by mouth. She was admitted at 5:00 P.M. Blood pressure was 120/82 and rose to a peak of 140/90 during labor. The blood sugar level was 69 mg. per 100 ml. The patient was given small doses of Seconal for sedation as labor gradually progressed. At 10:20 P.M. the cervix was thought to be

fully dilated. At 10:35 P.M. she was delivered spontaneously, under pudendal block, of a live 2,590-gram male infant in excellent condition. Immediate postpartum blood pressure was 120/90.

The puerperium was entirely uneventful. On October 1 the usual cortisone and sodium chloride dosage was resumed, and the patient became completely ambulatory that day. Attempts at breast feeding were only partially successful and the infant was given formula feeding before discharge. Mother and son were sent home on October 8 in excellent health. Albuminuria gradually waned until, on October 21, there was only a faint trace of albumin.

At the follow-up visit on November 12, the patient stated she had been "below par" after returning home from the hospital, which she attributed to the work of caring for the infant. The patient had had a two-day period of vaginal bleeding on October 28 and 29. Blood pressure was 105/75 and there was no albumin in the urine. Pelvic examination was within normal limits. Pubic hair had decreased in amount. On November 13, an oral supplement of 2 mg. of DCA twice a day was started and this improved matters.

Mrs. N. was seen again in April 1954, in the out-patient department. She was no longer taking DCA and she was well. In July of 1954, during a several-day period of severe heat, the patient complained of weakness and anorexia but failed to increase her salt intake. She was found dead in bed at home on the morning of July 14, 1954.

Autopsy was performed by Dr. Ralph Nelson of the Department of Pathology, University of Minnesota. There was no evidence of tuberculosis. The adrenal glands were not found at gross examination. The suprarenal fat on each side was examined microscopically and scarred remnants of one adrenal found (Fig. 1). The blood sodium level at autopsy was 128 mEq./L. The final diagnosis was idiopathic Addison's disease.

Laboratory methods[2]

Blood glucose concentration was determined by a modified Benedict's method (normal values, 70 to 90 mg. per 100 ml.); urea nitrogen by the urease method; chloride by the method of Schales and Schales; carbon-dioxide combining power by gas analysis in the Van Slyke-Neill apparatus after exposure to alveolar air; sodium and potassium by flame photometry; total protein by the biuret method; and fractionation of albumin and globulin by the Milne method using 26.8 per cent sodium sulfate.

Corticoids were measured by the hydrolysis of aliquots of 24-hour urine collections with acid at pH 1 and β-glucuronidase at pH 5. The residues were extracted with chloroform (3). 17-Ketosteroid determinations were performed by hydrolyzing the urines at

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[2] Determinations of blood glucose, urea nitrogen, chloride, carbon-dioxide combining power, sodium and potassium, total and fractional protein and pH were made in the laboratories of the Department of Laboratory Medicine.

The urinary corticoids, 17-ketosteroids, and pregnanediol determinations were carried out by Dr. Saul L. Cohen, Associate Professor of Physiological Chemistry, University of Minnesota.

The plasma progesterone determinations was made by Dr. Thomas R. Forbes, Department of Anatomy, Yale University.

The placental progesterone was measured by Dr. William H. Pearlman, Associate Professor of Biochemistry, the Jefferson Medical College of Philadelphia.

The 17-hydroxycorticosteroids were determined by Dr. Edmund B. Flink.

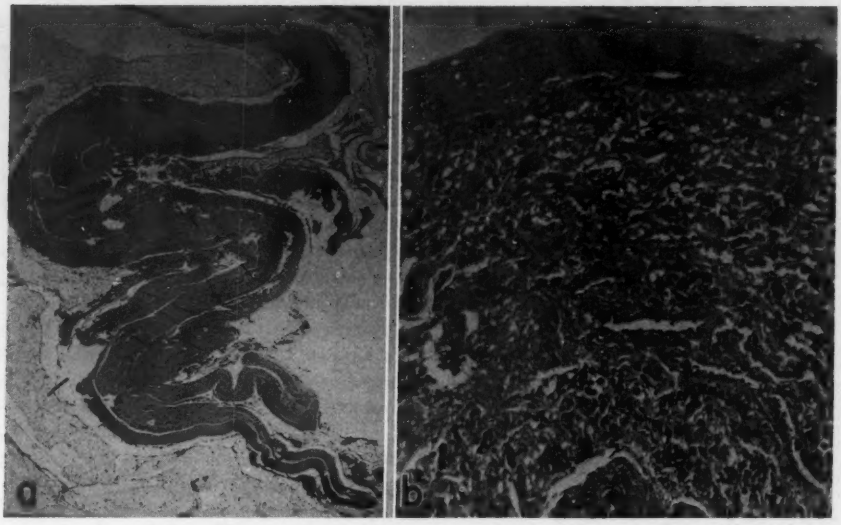

FIG. 1. **a.** Low-power view of section of the suprarenal fat, showing the much disrupted remnant of adrenal tissue found. At least half of this tissue is fibrous scar. **b.** High-power view of area outlined in **a.** There is no cortical tissue; the medulla is disorganized.

pH 5 by the method of Cohen (4). The ketonic fractions were prepared by the method of Bitman and Cohen (5) and assayed by the method of Holtorff and Koch (6).

Pregnanediol was determined by β-glucuronidase hydrolysis of the urine, followed by toluol extraction. The pregnanediol was then precipitated and assayed colorimetrically with sulfuric acid (7).

Both free and bound plasma progesterone levels were determined by the method of Hooker and Forbes (8, 9). The placental progesterone was measured by the method of Pearlman and Thomas (10).

Maternal serum iron concentration was measured by a modified Barkan and Walker technique (11).

Plasma 17-hydroxycorticosteroid levels were determined by the method of Nelson and Samuels (12). Urinary levels were estimated by the method of Reddy, Jenkins and Thorn (13).

Blood pH was measured in the Cambridge research model pH meter using a glass electrode with phthalate and phosphate buffers at 37° C.

RESULTS

Values for the various analyses of blood are shown in Table 1. When the initial determinations of electrolytes were made at the tenth week of pregnancy, the blood sodium and chloride levels were both depressed. This may have been related to the nausea and vomiting of early pregnancy. In any event, these aberrations vanished without additional therapy and

TABLE 1. BLOOD CHEMICAL VALUES IN A PREGNANT ADDISONIAN

Date (in 1953)	Feb. 24	Mar. 24	Apr. 23	May 21	June 18	July 9	July 30	Aug. 27	Sept. 17	Oct. 15	Nov. 16
Week of pregnancy	10	14	18	22	26	*29	32	36	39	−2 PP*	−6 PP
Glucose (mg./100 ml.)	83	74	65	68	71		67	63	64	78	81
Urea nitrogen (mg./100 ml.)	21	17	14	16	15		16	11	12	31	22
Chloride (mEq./L)	98	110		106		106	108	109	107	103	113
CO_2 comb. power (mEq./L)	22	21	21	20	19	16	19	19	20	21	26
Sodium (mEq./L)	132	138	136	141	137	144	136	145	146	136	144
Potassium (mEq./L)	5.8	5.5	4.6	4.4	4.4	4.3	4.4	4.4	4.5	5.9	4.5
Albumin (Gm. %)	1.2	3.6	2.9	3.7	3.0	2.6	3.4	2.8	2.2	2.7	3.9
Globulin (Gm. %)	6.9	3.6	4.0	3.0	3.4	3.6	3.2	3.3	3.4	3.8	2.6
Total protein (Gm. %)	8.1	7.2	6.9	6.7	6.4	6.2	6.6	6.1	5.6	6.5	6.5

* Post partum.

thereafter electrolyte homeostasis seemed to be well maintained (Fig. 2).

At the twenty-ninth week, the carbon-dioxide combining power had dropped to 16 mEq./L without significant change in chlorides, suggesting the possibility of an uncompensated metabolic acidosis in excess of that observed in normal pregnancy. When blood pH was determined simultaneously with CO_2 combining power three weeks later, the pH was 7.35 and CO_2 19 mEq./L.

The gradual drop of serum albumin from 3.4 Gm. per cent at the thirty-second week, to 2.2 Gm. per cent at the thirty-ninth week, during the period of albuminuria, may be meaningful, especially in view of the subsequent postpartum rise to 2.7 Gm. and then 3.9 Gm. per cent at six weeks

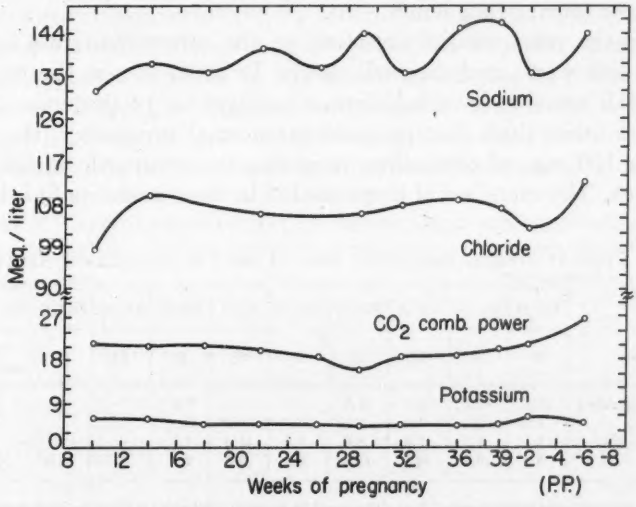

FIGURE 2

after delivery. There is no explanation for the initial value observed at the tenth week, of 1.2 Gm. per cent of albumin and 6.9 Gm. per cent of globulin. This 1:5.8 ratio had shifted markedly by the fourteenth week.

Because of the large volume of blood required for these procedures, some concern was felt about a possible iron deficiency anemia from blood loss. Hemoglobin concentration at the tenth week was 13.2 Gm. per cent. At the eighteenth week it was 10.8 Gm. per cent with a serum iron level of 70 gamma per 100 ml. At the thirty-second week it was 11.8 Gm. per cent with a serum iron level of 60 gamma per 100 ml. At the thirty-ninth week it was 12.0 Gm. per cent.

The values for urinary steroids are listed in Table 2 and shown graphically in Figure 3. It will be noted that the course of urinary excretion of corticoids was gradually downward, whereas the 17-ketosteroid excretion did not change appreciably. Through a laboratory error, corresponding determinations on urine samples for the final six weeks and for the initial postpartum visit were not made.

A blood sample drawn twenty-one hours after the last dose of 12.5 mg. of cortisone at the fourteenth week contained 6 gamma of 17-hydroxycorticosteroids per 100 ml. On the same day, four hours after cortisone, the level was 24 gamma per 100 ml. Urinary excretion on the same day was 3.8 mg. of 17-hydroxycorticoids per twenty-four hours. At the twenty-fourth week and the thirty-seventh week, the blood levels of 17-hydroxycorticosteroids four hours after cortisone were 27 gamma and 20 gamma per 100 ml., respectively. At the twenty-fourth week urinary excretion was 10.2 mg. per twenty-four hours.

The urinary pregnanediol excretion on the other hand rose until the twenty-fourth week, and then fell slowly. In order to test the possibility that this fall was due to metabolism of endogenous progesterone through some route other than that assumed for normal pregnancy, the patient was given 100 mg. of crystalline progesterone by mouth at the thirty-fourth week. The excretion of pregnanediol in the succeeding five days was

TABLE 2. URINARY STEROID EXCRETION (MG./24 HRS.) IN A PREGNANT ADDISONIAN*

Date (in 1953)	Feb. 24	Mar. 24	Apr. 23	May 21	June 18	July 9	July 30	Aug. 12	Sept. 17	Oct. 15	Nov. 16
Week of pregnancy	10	14	18	24	26	29	32	34	39	Post partum	Post partum
Corticoids (glucuronidase)	25.6	15.5	18.0	10.4	9.9	9.3	7.6				19.1
Corticoids (pH 1)	1.01	1.3	1.4	0.78	0.8	0.59	0.61				
17-Ketosteroids (HCl)	5	3.7	3.4	3.9	5.0	11.4	4.5				4.4
Pregnanediol	5.5	10.1	10.5	11.3	9.5	8.3	8.5	7.5	3.3	4.5	2.6

* The dose of cortisone during pregnancy was 12.5 mg. daily, except for an experimental period at the thirty-fourth week.

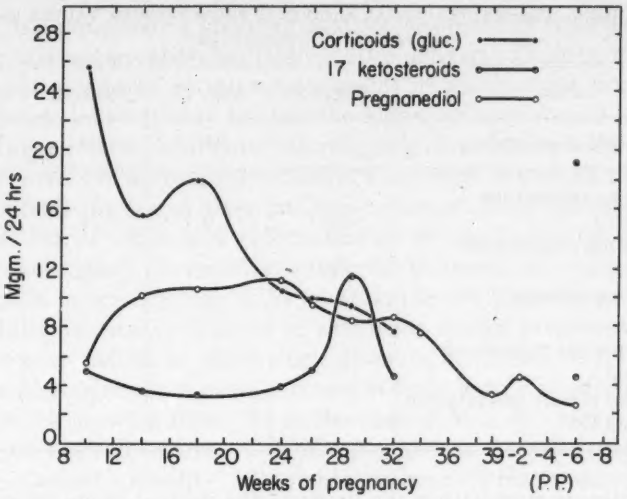

FIGURE 3

considerably augmented (Table 3), followed by a return to pre-medication levels.

The plasma progesterone levels in the last six weeks of pregnancy are presented in Table 4.

DISCUSSION

It has already been observed by others that the clinical course of Addison's disease which has been adequately stabilized by cortisone therapy in the non-pregnant state is in no way adversely affected by pregnancy. Further, there is nothing to suggest that cortisone administration affects the pregnancy itself adversely under these conditions (14–19).

The values obtained for blood hydroxycorticosteroids and urinary hy-

TABLE 3. URINARY PREGNANEDIOL EXCRETION AFTER PROGESTERONE LOADING

Date	Pregnanediol (mg./24 hrs.)
Aug. 13	6.5
Aug. 16, *100 mg. progesterone (oral)*	
Aug. 17	11.1
Aug. 18	22.2
Aug. 19	12.0
Aug. 20	9.5
Aug. 21	4.7

TABLE 4. PLASMA, PLACENTAL AND FETAL PROGESTERONE VALUES IN A PREGNANT ADDISONIAN

Date	Aug. 12	Aug. 27	Sept. 17	Sept. 30
Week of pregnancy	34	36	39	Delivery
Free plasma progesterone (γ/100 ml.)	<0.1	0.7	1.1	<0.1
Bound plasma progesterone (γ/100 ml.)	<0.1	<0.1	<0.1	<0.1
Placenta progesterone (γ/Gm.)				0.8
Fetal free plasma progesterone (γ/100 ml.)				0.5
Fetal bound plasma progesterone (γ/100 ml.)				0.1

droxycorticoids were within the limits of the normal range for pregnancy as reported by others (20–22).

The gradual but dramatic reappearance of Addisonian symptoms in Mrs. N. when cortisone was withheld at the thirty-fourth week of pregnancy indicates that, at least in her case, the steroid production of the fetus and placenta was not adequate replacement therapy (1). As reported previously (2), a study of circulating eosinophils at this time indicated the same lack of the physiologic diurnal rhythm as had been noted in Mrs. N. prior to pregnancy.

The low urinary excretion values for corticoids, 17-ketosteroids and pregnanediol as pregnancy advanced are also in keeping with the interpretation that the fetal-placental system did not substitute adequately for the known adrenal deficiency. Hills *et al.* (15), who studied 2 pregnant patients with surgically produced adrenal deficiency, arrived at a similar conclusion. Their patients both exhibited the more commonly observed increases in urinary excretion of neutral reducing lipids and 17-ketosteroids as the pregnant state advanced. The authors nevertheless conclude that these observations may be misleading, since such rises may not actually reflect an increase in the secretion of alpha-ketolic steroids. They further suggest that if secretion of alpha-ketolic steroids increases, then the metabolic demand must do so proportionately, since the patients remain vulnerable to adrenal insufficiency. The finding in the case of Mrs. N., that the glucuronidase corticoids actually fell and the 17-ketosteroids did not rise as pregnancy advanced, is compatible with the conclusion that there is no notable increase in secretion of steroids with adrenocortical activity in pregnancy. It suggests further than an increase in adrenocortical metabolic demand is not an inherent part of the pregnant state.

Mrs. N. is unique among pregnant Addisonians recently reported in that she died in a stress situation ten months after delivery. There is, therefore, available autopsy proof of the total absence of recognizable functioning adrenocortical tissue. It may be that the higher urinary steroid excretion observed in some other Addisonians during pregnancy has been the product of small remnants of adrenal cortex. Jailer attempted to exclude this possibility by submitting one of his patients to testing with corticotropin in the puerperium (23, 24). Hills *et al.* (15) carried out similar testing over a longer period but noted that the response of adrenal remnants to very prolonged stimulation in pregnancy may differ from that in the test situation.

The Addisonian patient's sense of well being during pregnancy on the other hand may well be on psychologic grounds, buttressed as it is by objective manifestations such as an increase in body hair, a greater work capacity and the growing fetus. As in the case of Mrs. N., many of these women have been amenorrheic, debilitated and on occasion gravely ill. The return toward normality, followed by pregnancy, may produce an impression of good health which is real but in fact delicately maintained. Mrs. N.'s deterioration when medication was withheld, and her subsequent unexpected death during a spell of hot weather, argue against a sense of false security based either on cortisone therapy or the pregnant state.

The observations on Mrs. N.'s urinary pregnanediol excretion are most intriguing from a study standpoint. It has long been supposed that the greatly increased urinary pregnanediol excretion in the pregnant state is a consequence of metabolism of greatly increased amounts of progesterone, produced in the maternal-placental system, to this end-product. Indeed, some of the vigorous efforts at hormonal therapy of abnormalities in pregnancy have rested on this assumption of augmented progesterone secretion. The observation that there is relatively little progesterone demonstrable in either maternal blood (25) or in the placenta itself (26, 27) is not in agreement with this conclusion. This has suggested that pregnanediol in pregnancy at least is the end-product of steroids other than progesterone, which may or may not be biologically active, secreted by either maternal adrenal cortex or placenta in increasing amounts.

Despite the unquestionable normality of Mrs. N.'s pregnancy, her urinary pregnanediol excretion was within the lower limits of normal in early pregnancy and thereafter was well below these limits, in a range ordinarily regarded as pathologic (28). Her ability to metabolize progesterone to pregnanediol was demonstrated at the thirty-fourth week. Indeed, the recovery of progesterone as pregnanediol during this test was greater than that ordinarily expected (29). The levels of plasma progesterone in maternal and fetal bloods were within the limits of normal for the Hooker-Forbes test, as noted. Placental progesterone content was slightly below the range of normal established by Pearlman and Cerceo (27). These find-

ings indicate that in this normal pregnancy, there was no remarkable augmentation in the production of any steroid excreted as pregnanediol after the twenty-fourth week. Since the patient had no adrenocortical tissue, the small increases which did occur must have come from substances secreted by the corpus luteum or placenta, which may or may not have been progesterone. This implies that the large increases observed in fully normal pregnancy are the result of metabolism of substances other than progesterone which are adrenocortical in origin.

SUMMARY

A patient with long-standing Addison's disease was studied during and after a normal pregnancy. The diagnosis was confirmed by the failure to find at autopsy any recognizable adrenocortical tissue.

During therapy with a constant dosage of 12.5 mg. of cortisone a day, there were no serious disturbances of electrolyte homeostasis during pregnancy.

Urinary corticoids, 17-ketosteroids and pregnanediol excretions were at relatively low levels in the second half of gestation. During a test period off therapy, symptoms of adrenal insufficiency developed. It is likely that pregnancy does not protect against adrenal insufficiency, despite the observation in other pregnant Addisonians of increased steroid excretion. Such increases are not requisite for a normal pregnancy.

Although pregnanediol excretion was low, the patient's plasma level and the placental and fetal progesterone levels were within or near normal limits. Furthermore, following a test dose of progesterone orally, she excreted large quantities of pregnanediol. This suggests that the pregnanediol excretion observed in normal pregnancy is the result of the increased secretion of steroids other than progesterone, which may or may not be biologically active, of adrenocortical origin.

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17-ALPHA-HYDROXYPROGESTERONE AND THE VIRILISM OF THE ADRENOGENITAL SYNDROME ASSOCIATED WITH CONGENITAL ADRENOCORTICAL HYPERPLASIA—A REVIEW

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IN 1941, Pfiffner and North (1, 2) and von Euw and Reichstein (3) isolated from the adrenal glands of animals the compound now described by the terminology of Fieser and Fieser (4) as 17-alpha-hydroxyprogesterone (originally designated as 17-beta-hydroxyprogesterone by the nomenclature current at the time of isolation). The biologic activity of this compound has been studied in animals by several investigators. According to Pfiffner and North (1, 2), this steroid has a weak and ineffective progestational action; their findings have been confirmed by Junkmann (5). Pfiffner and North (1) claimed that 17-alpha-hydroxyprogesterone has a slight mineralocorticoid action. These same workers reported (2) that the compound has no corticoid activity by the survival test in immature adrenalectomized male rats. Ingle (6) also found that the steroid has no corticoid action by the work test in adult adrenalectomized male rats. The conflicting reports (1, 2, 5, 7–9) concerning the androgenic activity of 17-alpha-hydroxyprogesterone will be described in detail later.

Recently, attention has been focused on the biologic significance of 17-alpha-hydroxyprogesterone by two developments: 1) the suggestion that this steroid plays an important role as an intermediate in the biosynthesis of cortical hormones by the adrenal glands, and 2) the proposal that a disturbance in the intermediary metabolism of this steroid accounts for certain manifestations of the adrenogenital syndrome associated with congenital adrenocortical hyperplasia. It is the purpose of this discussion to consider some of the evidence concerning this second relationship.

Hechter and associates and Hayano and Dorfman (10–12) have proposed a schema for the biosynthesis of 17-hydroxycorticosterone (hydrocortisone, compound F) by the adrenal glands. The steps include the conversion of progesterone into 17-alpha-hydroxyprogesterone, and then the conversion of this latter substance into hydrocortisone (Fig. 1). From studies in which progesterone was perfused through beef adrenal glands,

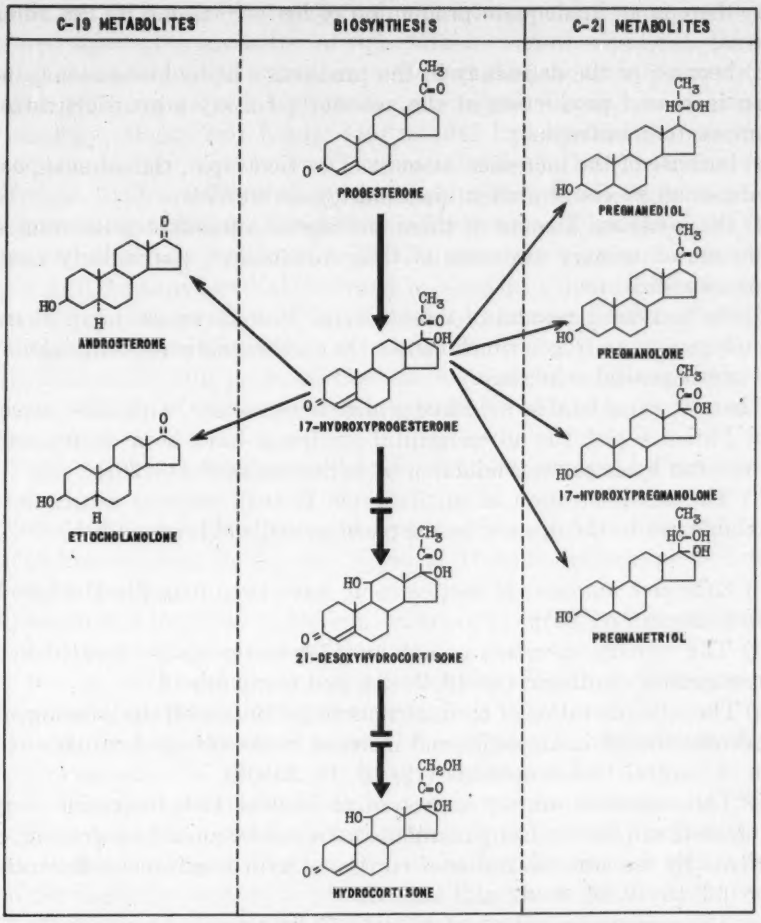

FIG. 1. Diagram to illustrate the relationship of 17-alpha-hydroxyprogesterone to the biosynthesis of hydrocortisone by the adrenal glands, to the production of C-19 and C-21 urinary steroid metabolites, and to the defects (broken arrows) that are postulated in the adrenogenital syndrome in the enzymatic hydroxylation mechanisms of the adrenal glands for the conversion of this steroid and/or 21-desoxyhydrocortisone into hydrocortisone. [Adapted from Jailer *et al.* (34)].

these investigators reported a 3 to 7 per cent conversion into 17-alpha-hydroxyprogesterone, and a 10 to 12 per cent conversion into 17-alpha-hydroxycorticosterone (hydrocortisone).

In 1951, Bartter and associates (13) postulated that the sequence of events in the pathologic physiology of the adrenogenital syndrome associated with congenital adrenal hyperplasia is as follows:

1) there is an inadequate production of hydrocortisone by the adrenal glands;

2) because of the deficiency in the production of hydrocortisone, there is an increased production of the anterior pituitary adrenocorticotropic hormone (corticotropin);

3) because of the increased amount of corticotropin, the adrenal cortex produces an excess of unidentified androgenic steroids;

4) the increased amount of these androgenic adrenal steroids results in an increased urinary excretion of their metabolites, particularly neutral 17-ketosteroids; and

5) the increased amount of these adrenal steroids results in an increase in endogenous androgen which causes the characteristic masculinization of the adrenogenital syndrome.

There is considerable evidence which is consistent with this concept:

a) Patients with the adrenogenital syndrome have been demonstrated to have an inadequate production of hydrocortisone (13–20).

b) The administration of corticotropin to such patients causes no appreciable rise in the urinary or the plasma corticoid levels (13, 15, 16, 20–22).

c) Excessive amounts of corticotropin have been found in the blood of such patients (20, 23);

d) The urinary excretion of neutral 17-ketosteroids is elevated in the adrenogenital syndrome (13–19, 24–34, and many others).

e) The administration of corticotropin to patients with the adrenogenital syndrome results in an additional increase in the elevated urinary excretion of neutral 17-ketosteroids (13, 16, 18, 35–38).

f) The excessive urinary excretion of neutral 17-ketosteroids can be eliminated and the virilizing manifestations can be made to regress in such patients by the administration of cortisone, hydrocortisone or fluorocortisone (13, 15–19, 34, 39–42, and others).

g) The urinary excretion of neutral 17-ketosteroids tends to be high when corticotropin production appears to be high (as in Cushing's syndrome associated with bilateral adrenocortical hyperplasia), and tends to be low when corticotropin production appears to be low (as in Cushing's syndrome associated with an adrenocortical adenoma (43)). In the latter condition, the contralateral adrenal gland usually is atrophied. This is interpreted to indicate that there has been inhibition of corticotropin production by the excessive amount of hydrocortisone that is elaborated autonomously by the adenoma without the necessity of corticotropin stimulation.

In 1937, Butler and Marrian (44) described the presence of pregnanetriol in the urine of 2 women with the adrenogenital syndrome. Subsequently, Mason and Kepler (45) isolated the same compound from the urines of

female patients with this condition, and Miller and Dorfman (46) discovered significant quantities of this steroid in another patient. In 1953, Bongiovanni (47) called attention to the fact that the urinary excretion of pregnanetriol regularly is high in the adrenogenital syndrome. Reasoning by analogy, Mason and Kepler (45) in 1945 had reached the conclusion that pregnanetriol must be a urinary metabolite of 17-alpha-hydroxyprogesterone. Evidence that the interpretation of Mason and Kepler is correct was presented in 1954 by Bongiovanni, Eberlein and Cara (18), who demonstrated that the administration of 17-alpha-hydroxyprogesterone to patients with the adrenogenital syndrome resulted in an increase in the urinary excretion of pregnanetriol. This observation has been confirmed recently by Jailer and associates (34).

In 1953, Jailer (16) proposed that the sequence of events in the pathologic physiology of the adrenogenital syndrome should be revised as follows:

1) there is an inadequate production of hydrocortisone by the adrenal glands;

2) the inadequate production of hydrocortisone by the adrenal glands results from a block in the conversion of 17-alpha-hydroxyprogesterone into hydrocortisone;

3) because of the block in the conversion of 17-alpha-hydroxyprogesterone, an excess of this steroid accumulates;

4) because of the deficiency in the production of hydrocortisone, there is an increased production of corticotropin;

5) because of the increased amount of corticotropin, the adrenal cortex produces an excess of 17-alpha-hydroxyprogesterone;

6) an increased amount of 17-alpha-hydroxyprogesterone results, therefore, both from the excessive accumulation of the steroid because of the block to its conversion into hydrocortisone by the adrenal glands, and from the excessive production of this steroid by the adrenal glands;

7) the increased amount of 17-alpha-hydroxyprogesterone results in an increased urinary excretion of its metabolites, particularly pregnanetriol and neutral 17-ketosteroids; and

8) the increased amount of 17-alpha-hydroxyprogesterone results in an increase in endogenous androgen, which causes the characteristic masculinization of the adrenogenital syndrome.

In a subsequent communication, Jailer *et al.* (34) amplified this concept. They postulated that in the adrenogenital syndrome there are defects in the enzymatic hydroxylation mechanisms of the adrenal glands which are essential for the conversion of 17-alpha-hydroxyprogesterone and/or 21-desoxyhydrocortisone into hydrocortisone. Thus partially hydroxylated hydrocortisone-precursors escape into the circulation and are metabolized

along well known pathways, resulting in the production of androgens (Fig. 1).

The evidence that favors the alterations proposed by Jailer and associates (16, 32) in the sequence of events in the pathologic physiology of the adrenogenital syndrome includes the following points:

a) The urinary excretion of pregnanetriol is elevated in patients with the adrenogenital syndrome (18, 19, 34, 38, 44–48).

b) The administration of corticotropin to patients with this disorder induces an additional increase in the elevated urinary excretion of pregnanetriol which parallels the rise in the elevated urinary excretion of neutral 17-ketosteroids (18, 38, 49).

c) The excessive urinary excretion of pregnanetriol in patients with the adrenogenital syndrome can be suppressed by the administration of cortisone (18, 19, 47).

d) The administration of moderate and large amounts of 17-alpha-hydroxyprogesterone to human beings results in the urinary excretion of pregnanetriol, regardless of the status of adrenocortical function (34, 49–51). Langecker and Prescher (50) obtained a definite increase in the urinary pregnanetriol excretion of a healthy male subject following the intramuscular injection of 1 gram of 17-alpha-hydroxyprogesterone (Fig. 2). In a subsequent study in which another normal male subject received an intramuscular injection of 1 gram of the steroid, Langecker (49) found that considerable amounts of pregnanetriol could be identified when the urine was hydrolyzed with the enzyme, beta glucuronidase (Fig. 3). Jailer and associates (34) administered 17-alpha-hydroxyprogesterone to 8 patients orally, as a water suspension or in gelatin capsules. The total amount administered varied from 600 to 1,000 mg. in divided doses over a two-day or three-day period. At the time of administration, all of the patients were excreting minimal amounts of endogenous 17-ketosteroids. Four of the patients had adrenocortical insufficiency (2 cases of hypopituitarism; 1 of Addison's disease being treated with desoxycorticosterone acetate, and 1 of Addison's disease being treated with cortisone), and 4 had the adrenogenital syndrome with adrenocortical hyperplasia (all being treated with cortisone). All of the patients showed a significantly increased urinary excretion of pregnanetriol after the administration of the 17-alpha-hydroxyprogesterone. Bongiovanni *et al.* (51) recovered and identified pregnanetriol from the urine of a 10-year-old boy with Addison's disease to whom 17-alpha-hydroxyprogesterone had been administered parenterally.

e) The administration of 17-alpha-hydroxyprogesterone in quantities that are not sufficient to induce a urinary excretion of pregnanetriol in subjects with normal adrenocortical function results in a rise in the ele-

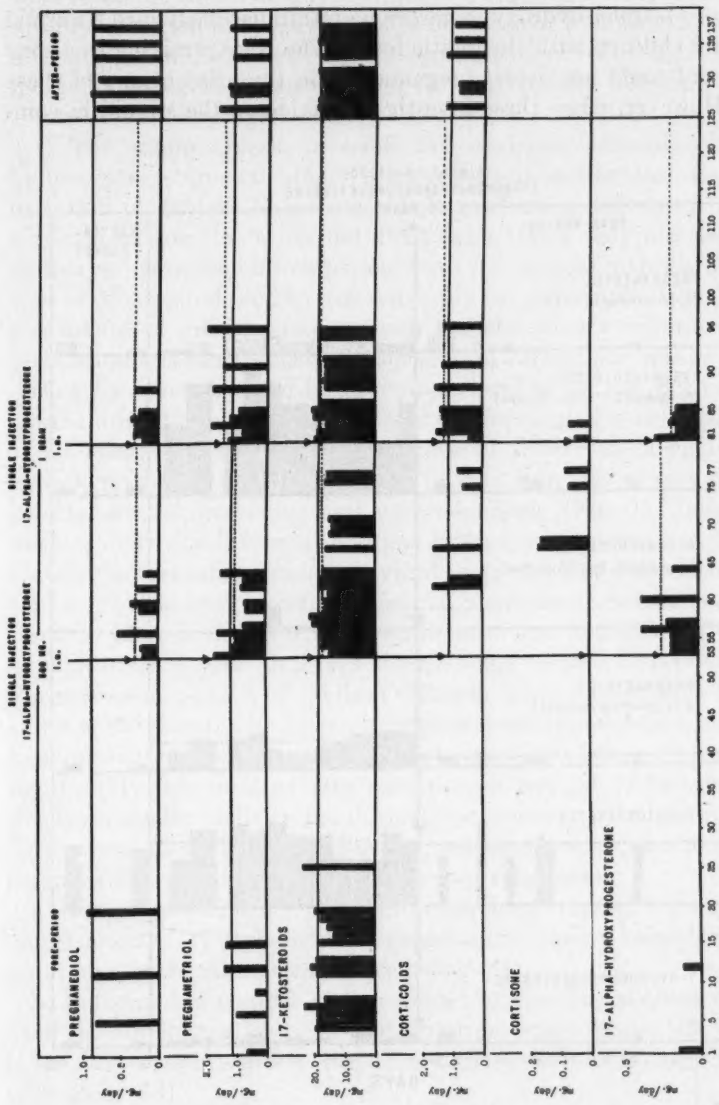

FIG. 2. The effect of two single intramuscular injections of 17-alpha-hydroxyprogesterone on the urinary secretion of steroid metabolites by a healthy adult male subject. [Adapted from Langecker and Prescher (50).]

vated urinary excretion of this metabolite in patients with the adrenogenital syndrome (18). Bongiovanni, Eberlein and Cara (18) injected 25 to 50 mg. of 17-alpha-hydroxyprogesterone intramuscularly into 2 normal adults and 2 children with rheumatic fever (who were receiving cortisone therapy), and could not detect pregnanetriol in the urine of any of these patients. However, when these investigators injected the steroid in com-

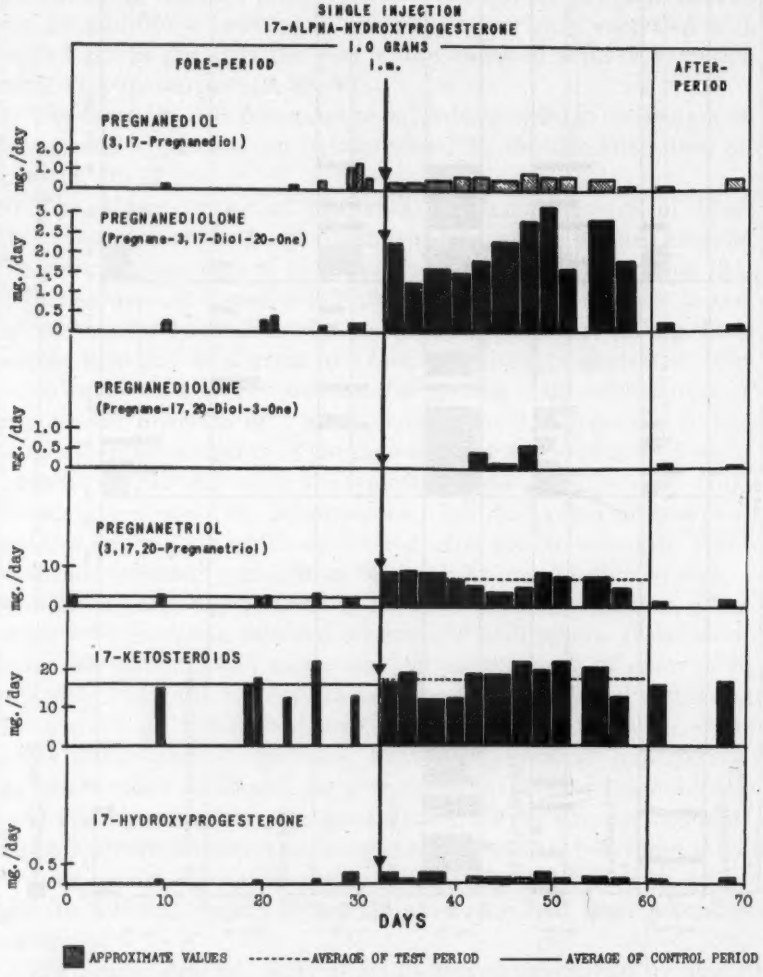

FIG. 3. The effect of a single intramuscular injection of 17-alpha-hydroxyprogesterone on the urinary excretion of steroid metabolites by a healthy adult male subject. [Adapted from Langecker (49).]

parable amounts into 3 patients with the adrenogenital syndrome (who were receiving cortisone therapy), there was a considerable increase in the urinary excretion of pregnanetriol in one patient following 25 mg., a definite increase in the excretion in the second patient following 50 mg., and a marked increase in the excretion in the third patient following 100 mg. of 17-alpha-hydroxyprogesterone.

f) The administration of small and moderate amounts of 17-alpha-hydroxyprogesterone to human beings results in an elevation in the urinary excretion of neutral 17-ketosteroids, regardless of the status of adrenocortical function (18, 34, 53, 54). Polley and Mason (53) injected 17-alpha-hydroxyprogesterone into a patient with rheumatoid arthritis in an initial dose of 50 mg., followed by 100 mg. daily for eight more days. There was no evidence of antirheumatic activity, but the urinary excretion of neutral 17-ketosteroids rose from an average level of 2.5 mg. per twenty-four hours during the control period to an average level of 4.5 mg. per twenty-four hours during the administration of the compound. Fukushima, Dobriner and Gallagher (54) showed that injected deuterium-labeled 17-alpha-hydroxyprogesterone is metabolized in man into the urinary neutral 17-ketosteroids, androsterone and etiocholanolone (Fig. 1). Jailer and associates (34) administered 17-alpha-hydroxyprogesterone orally over a two-day to three-day period in divided doses totalling 600 to 1,000 mg., to 8 patients who were excreting minimal amounts of endogenous 17-ketosteroids. Four of the patients had adrenocortical insufficiency (2 cases of hypopituitarism, 1 of Addison's disease being treated with desoxycorticosterone acetate, and 1 of Addison's disease being treated with cortisone), and 4 of the patients had the adrenogenital syndrome with adrenocortical hyperplasia (all being treated with cortisone). All of the patients showed a significantly increased urinary excretion of neutral 17-ketosteroids. By chromatographic technics and infrared spectrometry, the following metabolites were identified: androsterone, etiocholanolone, 3-alpha-hydroxy-pregnane-20-one, and 17-alpha-hydroxy-pregnanolone.

g) The pattern of urinary steroid metabolites excreted following the administration of 17-alpha-hydroxyprogesterone closely resembles the pattern found in the adrenogenital syndrome (34).

h) Some of the neutral 17-ketosteroid urinary metabolites that result from the administration of 17-alpha-hydroxyprogesterone (19, 34, 54), as in the case of androsterone (Fig. 1), are known to have biologic activity as androgens (55).

On the other hand, there are some observations that are difficult to interpret with the sequence of events proposed by Jailer and associates (34):

a) The administration of 17-alpha-hydroxyprogesterone to individuals

with normal adrenocortical function has not been followed by an increase in the production of hydrocortisone as indicated by the urinary excretion of corticoid metabolites (50, 53). Polley and Mason (53) observed no change in the urinary excretion of corticosteroids by a patient with rheumatoid arthritis following the injection of 17-alpha-hydroxyprogesterone in an initial dosage of 50 mg., followed by 100 mg. daily for eight more days. Langecker and Prescher (50) found no increase in the urinary excretion of corticoids and cortisone after the intramuscular injection of 1 gram of 17-alpha-hydroxyprogesterone into a healthy male subject (Fig. 2). However, 17-alpha-hydroxyprogesterone apparently can be converted into hydrocortisone only in the adrenal gland, whereas it can be converted into pregnanetriol or neutral 17-ketosteroids in extra-adrenal sites as well as in the adrenal gland. Therefore, if the adrenal alone is to produce a recognizably increased amount of hydrocortisone, the concentration of 17-alpha-hydroxyprogesterone in the blood perfusing the gland would have to be higher than the concentration of 17-alpha-hydroxyprogesterone in the blood perfusing the several sites of conversion if they are to produce increments of steroid materials which would amount to a recognizable increase in either pregnanetriol or neutral 17-ketosteroids. Hence, the difficulty in demonstrating an increase in hydrocortisone following the administration of 17-alpha-hydroxyprogesterone is not necessarily a point against the sequence of events proposed by Jailer. No reports have been found on the effect of 17-alpha-hydroxyprogesterone on hydrocortisone production in patients with the adrenogenital syndrome.

b) Although an increased urinary excretion of neutral 17-ketosteroids occurs in human beings, regardless of the status of the adrenocortical function, following the administration of small and moderate quantities of 17-alpha-hydroxyprogesterone (18, 34, 53, 54), the injection of large amounts of this steroid into subjects with normal adrenocortical function is not followed by an increase in the urinary excretion of these steroid metabolites (49, 50). Langecker and Prescher (50) were not able to demonstrate a significant increase in the urinary excretion of neutral 17-ketosteroids by a healthy male subject following the intramuscular injection of 1 gram of 17-alpha-hydroxyprogesterone (Fig. 2). Langecker (49) repeated the study in another normal male subject, and again with more elaborate technics could not obtain a significant increase in the urinary excretion of 17-ketosteroids following the intramuscular injection of 1 gram of the steroid (Fig. 3).

c) The biologic activity of 17-alpha-hydroxyprogesterone as an androgen is not established (1, 2, 5, 7–9). There are conflicting reports concerning the androgenic action of 17-alpha-hydroxyprogesterone in animals. Pfiffner and North (1) reported that by injection the steroid had an androgenic

effect on castrated infantile rats, and stimulated the growth of the seminal vesicles and prostate. In contrast, Junkmann (5) was not able to demonstrate an androgenic action in normal infantile male rats (weighing 35 to 45 grams) with subcutaneous injections of up to 1 mg. of the compound daily for fourteen days. Pfiffner and North (2) reported that 17-alpha-hydroxyprogesterone by injection had no androgenic effect on the chick's comb. Butenandt (7) administered the steroid intramuscularly into capons, and reported that it was about half as effective as androsterone in stimulating the growth of the comb; he also observed an androgenic effect on the capon comb following local administration. Dorfman (8) found that 17-alpha-hydroxyprogesterone had no consistent androgenic action. By direct application to the chick's comb, 80-microgram and 640-microgram doses were without effect, whereas a 320-microgram dose induced a 29 per cent comb ratio increment which was statistically significant. His data indicated, however, that at this dosage level the steroid by local application was less than one-thirtieth as effective as androsterone by direct application. Dorfman reported that when 17-alpha-hydroxyprogesterone was injected subcutaneously in a dosage of 1,000 micrograms, there was significant comb inhibition. Gassner (9) found that the steroid had practically no androgenic activity locally when applied to the cock's comb, but that it had slightly less than half of the androgenic action of methyltestosterone when administered orally to cockerels. No mention has been made of the development of masculinization in human beings following the administration of 17-alpha-hydroxyprogesterone by any of the investigators who have given the steroid (16, 18, 34, 49–54); however, their studies were not designed specifically to examine the androgenic action of the compound. No investigations that were deliberately planned to determine the androgenicity of the steroid in man have been reported. Only indirect evidence has been supplied by the studies in man on the urinary steroid metabolites that are excreted following the administration of 17-alpha-hydroxyprogesterone (19, 34, 54). Because this compound produces some metabolites (such as androsterone) which are known to be androgenic (55), it has been postulated by Jailer and associates (34) that an excess of 17-alpha-hydroxyprogesterone will result in masculinization. However, the androgenic activity of this steroid in man is still to be established.

d) In some patients with the adrenogenital syndrome, the block to the production of hydrocortisone appears to be chiefly in the conversion of 21-desoxy-hydrocortisone rather than in the conversion of 17-alpha-hydroxyprogesterone (18, 34, 56, 57). In order to explain the development of the masculinization by the mechanism of Jailer and associates (34), it is necessary to assume that in these cases 21-desoxy-hydrocortisone rather than 17-alpha-hydroxyprogesterone is the steroid that results in an increased

amount of endogenous androgen and masculinization. The biologic activity of 21-desoxy-hydrocortisone is not known. To explain the development of the virilism by the mechanism of Bartter and associates (13), it is not necessary to modify the sequence of events in these cases, since the patients with the block to the conversion of 21-desoxy-hydrocortisone have the same defect of inadequate production of hydrocortisone as do those with the block to the conversion of 17-alpha-hydroxyprogesterone.

SUMMARY

This discussion can be summarized by one question: is the virilism of the adrenogenital syndrome induced by an excess of 17-alpha-hydroxyprogesterone, as postulated by Jailer and associates (34), or is it induced by an excess of unidentified androgenic adrenal steroids, as proposed by Bartter and associates (13)? Dorfman (58) has suggested that the masculinization results from large amounts of C-19 androgens (such as Δ^4-androstene, 3,17-dione) which are synthesized by the adrenal glands from acetate or cholesterol but not by way of the C-21 precursors (such as 17-alpha-hydroxyprogesterone).

One of the important steps in answering this question is to determine whether or not 17-alpha-hydroxyprogesterone is a biologically active androgen when administered parenterally in man. This problem is now being studied by several investigators.

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Editorial

CUSHING'S SYNDROME—AN ADRENAL OR PITUITARY DISEASE?

IT IS generally agreed that the clinical picture of Cushing's syndrome is ascribable to hyperfunction of the adrenal cortex. All the signs, symptoms and metabolic characteristics of the disorder have been reproduced by the prolonged administration of cortisone or hydrocortisone. When the pathologic basis of the condition is found to be a benign or malignant tumor of the adrenal cortex, the increased secretion of "glucocorticoids" can be explained by analogy with tumors arising in certain other endocrine organs. However, the ultimate cause of that form of the disease which is associated with bilateral adrenocortical hyperplasia has so far escaped detection.

The three general hypotheses which have been advanced to explain this entity are: 1) basophilic adenoma with implied excessive secretion of corticotropin (1); 2) hypothalamic disease stimulating the pituitary to secrete corticotropin (2); 3) intrinsic adrenal disease with resultant increased sensitivity of the adrenal cortex to corticotropin (3–5). With respect to the first two hypotheses, there is evidence that both the changes in the basophilic cells of the pituitary (6) and histologic lesions in the hypothalamus (7) may actually be secondary to prolonged elevation of corticosteroid levels.

Data collected in this laboratory lend themselves to a working hypothesis which suggests a different concept of pituitary-adrenal relationships in this disease.

In 11 patients with Cushing's syndrome and adrenocortical hyperplasia, an exaggerated response of the gland to exogenous corticotropin (ACTH) has been demonstrated. A standardized intravenous corticotropin test (8) caused a rise of plasma 17-OH-corticosteroid levels to an average of 90 micrograms per 100 ml. Rises of the same order of magnitude had been shown earlier by Eik-Nes *et al.* (9), Grumbach *et al.* (5), and in this laboratory (8). This excessive response of the hyperplastic adrenal cortex was in contrast to the response of 40 normal subjects, in whom the same procedure caused elevation of plasma 17-OH-corticosteroid levels to an average of 46 micrograms per 100 ml. (10). The exaggerated adrenocortical reactivity in Cushing's syndrome does not appear to be due to an increase in adrenal

mass, since it was not appreciably reduced in patients who had undergone unilateral adrenalectomy. Furthermore, in 1 patient, both adrenal glands were found to be of normal weight.

In an attempt to assess the degree of "pituitary-dependence" of the hyperplastic adrenals, prednisone in a dosage of 30–50 mg. daily was administered to 6 of the patients over periods of seven to ten days. It had been shown earlier that amounts of prednisone as small as 20 mg. per day, administered to normal subjects over comparable periods, resulted in inhibition of the plasma 17-OH-corticosteroid response to exogenous corticotropin (10). Unlike the normal individuals, patients with Cushing's syndrome showed only minor diminution in the exaggerated adrenocortical response to corticotropin after administration of prednisone. Since 3 patients showed a fall in urinary 17-ketosteroid excretion during prednisone administration, it is inferred that this steroid has some inhibitory effect on the activity of the pituitary-adrenal system.

A third observation is thought to be relevant to the present problem. In 3 patients with rheumatoid arthritis, daily administration of corticotropin as ACTH "Gel" over periods of four to five years was not associated with an excessive rise of plasma 17-OH-corticosteroid levels after the standard corticotropin (ACTH) test. Adrenocortical responsiveness in these patients was well within the limits of normal.

At first glance one might conclude that these observations could be explained on the basis of intrinsic disease of the adrenal cortex. Such an explanation would conform with the view of Grumbach and his co-workers (5), who suggested that an increased quantity of an adrenal enzyme system involved in hydrocortisone biosynthesis might be the basic derangement in adrenal hyperplasia. This opinion, however, does not explain why procedures directed at reduction of anterior pituitary function favorably affect the course of this variety of Cushing's syndrome. Three lines of evidence are difficult to reconcile with the hypothesis that the seat of the disorder is primarily in the adrenal cortex. First, about 25 to 40 per cent of patients with the disease enjoy at least temporary remission as a result of pituitary irradiation (11, 12, 13) without demonstrating any of the clinical or biochemical features of hypopituitarism. Second, 3 patients who had received pituitary irradiation were subjected to standard corticotropin tests during periods of clinical remission (six months, and three and six years after treatment). Their 17-OH-corticosteroid responses were within normal limits or only very slightly exaggerated. In these patients, the various indices of adrenocortical function suggested that adrenal function had reverted nearly to normal as a result of the therapy. If the hyper-responsiveness found in active Cushing's syndrome were inherent in the adrenal, successful pituitary irradiation might well be expected to decrease the rate of

release of adrenocortical steroids by virtue of a fall in circulating corticotropin. However, administration of exogenous corticotropin should demonstrate a persistent adrenal abnormality despite apparent remission of the disease. Third, Arner *et al.* (14) showed that electrocoagulation of the anterior pituitary could induce a clinical remission of Cushing's syndrome associated with bilateral adrenal hyperplasia—again without causing evidence of hypopituitarism.

These observations rather point to the pituitary as the more likely site of the primary disorder.

If the pituitary plays a causative role, one would expect an excess of corticotropin to be the mechanism, as Sulman (15) and others have claimed. Certainly, the prolonged administration of corticotropin results in the clinical features of hyperadrenalism. Despite the apparent reasonableness of this hypothesis, the best evidence appears to be against it. Paris *et al.* (16) and Nelson (17), using two different technics, have been unable to detect excessive circulating corticotropin in patients with adrenal hyperplasia and Cushing's syndrome. However, these workers, like Taylor and his colleagues (18), have consistently found evidence of the hormone in patients with Addison's disease. Furthermore, as just noted, chronic administration of corticotropin failed to induce the hyper-response of the adrenal cortex which has been found to be such a constant feature of this type of Cushing's syndrome.

If, as is suggested, the pituitary can be incriminated as the primary cause of adrenal hyperplasia, how is the pathologic effect brought about? From the work of Reinhardt *et al.* (19) and Stack-Dunne and his collaborators (20), there is evidence pointing to the existence of a "corticotropic factor" distinct from either the ascorbic-acid-depleting factor or steroidogenic corticotropin. Liddle *et al.* (21) have shown that such a factor may potentiate the steroidogenic effect of "orthodox" corticotropin in human subjects. Such a potentiation could account for the hyper-responsiveness to corticotropin which is characteristic of Cushing's syndrome.

If one assumes that in Cushing's syndrome with bilateral adrenal hyperplasia the anterior pituitary secretes an excess of a "corticotropin-potentiating substance," many of the conflicting observations and interpretations of this disease could be harmonized. Such a postulate, for example, would explain the failure to find excess corticotropin, assayed by the *ascorbic-acid-depleting test* (16–18), in the blood of patients with Cushing's syndrome. One would have to assume that although potent cortical steroids (*e.g.*, prednisone) inhibit the pituitary release of "orthodox" corticotropin (10), they have less inhibitory effect in regulating pathologic pituitary secretion of "corticotropin-potentiating factor." This assumption would account for the apparent paradox of failure of prednisone to abolish or even

to diminish adrenocortical hypersensitivity to exogenous corticotropin (over the periods of time employed in this study), while at the same time causing a fall in urinary 17-ketosteroid excretion.

Finally, the postulate of a "corticotropin-potentiating" material of pituitary origin is in harmony with the demonstrated beneficial effects which follow a direct attack on the pituitary. The postulate would account for remission following partial hypophysectomy (14). If the hypothalamus or the anterior pituitary were assumed to be the source of the aberrant secretion, the occasional success of pituitary irradiation (11, 12, 13) would be explained by the fact that both areas are usually included in the fields irradiated. Cessation of excessive "potentiating factor" secretion would then in turn explain the loss of hyper-responsiveness to corticotropin in the 3 patients cited, in whom pituitary irradiation was followed by clinical remission. It is realized that the evidence given here is circumstantial. Until such time as the existence of a "corticotropin-potentiating factor" is demonstrated in the blood of patients with Cushing's syndrome, the foregoing concept must remain in the realm of speculation.

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Letters to the Editor

PLASMA 17-KETOSTEROIDS IN AN ANENCEPHALIC INFANT

To the Editor:

Postnatal involution of the adrenal glands in the early months of extrauterine life is unique in man and possibly in certain other primates and larger felidae of the order Carnivora. A decrease in both the size and the weight of the adrenals occurs which is entirely the result of involution of a specific portion of the cortex known as the transient, "fetal," or internal cortex (2). There have been many observations and much speculation recorded concerning the function of this tissue.

The daily urinary excretion of 17-ketosteroids and the level of 17-ketosteroids in the plasma are relatively high in the first week of life but decrease thereafter (3). Furthermore, the levels of neutral 17-ketosteroids are higher in the fetal than in the maternal circulation, as determined in plasma obtained from the umbilical cord and from pregnant women at the time of delivery. It has been suggested that the inner cortex of the fetal adrenal may be a source of these 17-ketosteroids (4, 5).

Adrenal hypoplasia of variable degree, but in some instances severe, is characteristically present in anencephalic monsters (6). The fetal cortex of these infants is usually absent or considerably reduced in size. If the sole source of 17-ketosteroids in the fetus were from this zone of the adrenals, diminished levels or absence of 17-ketosteroids might be expected in such infants. We are not aware of any previous measurements of the levels of any steroid in the plasma of anencephalic infants.

Recently, we had the opportunity of measuring the concentration of 17-ketosteroids in the plasma of a full-term anencephalic infant at 12 hours of age. The value which was obtained—279 micrograms per 100 milliliters—is higher than that found in normal adults. The method used was essentially that employed by Gardner (4); in this laboratory, the normal adult values are 44–117 (mean 74.5) micrograms per 100 milliliters of plasma. The infant died at 25 hours of age and postmortem examination revealed very small adrenal glands which together weighed only 0.75 gram. (The normal mean weight of the two adrenals in newborn infants of comparable size is 6.5 grams.) On histologic examination there was almost complete absence of the fetal cortex.

It is clear that the elevated level of plasma 17-ketosteroids found in this infant could not have been derived from the fetal adrenal cortex. Although their origin remains obscure, other possible sources include the outer or "adult" cortex (reticular zone?), the placenta, or the maternal adrenal cortex. In view of Gardner's observation (4) that the level of neutral 17-ketosteroids in the fetal circulation is higher than that in the maternal circulation, the placenta appears to be the most likely source of these steroids.

Recent studies (7, 8) on the detection and identification of C-19 steroids in human fetal adrenal glands from the ninth to the twenty-first week of gestation have shown a decrease of these steroids per gram of adrenal tissue with advancing fetal age. It has been suggested that C-19 steroid synthesis occurs in the fetal zone with decreasing activity as fetal age advances. These data are consistent with our contention that the elevated levels of plasma 17-ketosteroids in newborn infants are not derived from the fetal adrenal cortex.

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CONVERSION OF PROGESTERONE, 17-α-HYDROXY-PROGESTERONE AND 11-DEOXYCORTISOL TO CORTISOL BY THE HUMAN ADRENAL GLAND *IN VITRO*

TO THE EDITOR:

In 1951, Hechter *et al.* (1) reported on the biosynthesis of adrenocortical steroids by perfusion of the bovine adrenal gland *in vitro*. Since then several biosynthetic reactions on steroid substrates have been demonstrated by the same group using the perfusion technique. These include the oxidation of the Δ^5-3-hydroxyl group to Δ^4-3 ketone, 6β-hydroxylation, 11β-hydroxylation, 17α-hydroxylation, and 21-hydroxylation. The same reactions have been demonstrated in bovine adrenal enzyme preparations by Hayano and Dorfman, Plager and Samuels, Savard, Meyer, Haines, McGinty, and others (2). More recently, Bloch *et al.* (3) have shown the conversion of acetate to dehydroepiandrosterone by human adrenal gland slices from a patient with the adrenogenital syndrome. In this note, we report the conversion of progesterone, 17α-hydroxyprogesterone, and 11-deoxycortisol to cortisol by human adrenal tissue *in vitro*.

Adrenal glands obtained from female patients with metastatic carcinoma of the breast were used for this study. Adrenal slices were prepared from two glands weighing 7.68 and 8.39 Gm., respectively. A mince was prepared from a third gland weighing 5.62 Gm. These preparations were placed in a medium containing 25 ml. of Krebs-Ringer-phosphate (4) solution, 15 mg. of diphosphopyridine nucleotide, 1 ml. of 0.02 M sodium adenosine triphosphate in 80 per cent glycerol, and 1 ml. of 0.52 M sodium fumarate. The following substrates in approximately 0.3 ml. of 95 per cent ethyl alcohol were added to the three preparations respectively: deoxycortisol (5 mg.), 17α-hydroxyprogesterone (5 mg.), and progesterone (5 mg.). The incubations were begun within ten to twenty minutes after removal of the gland from the patient. All preparations were incubated for three hours at 37° C. with 95 per cent O_2–5 per cent CO_2 as the gas phase. The incubated samples were immediately set up for extraction with methylene chloride by a continuous dialysis and extraction technique previously described (5). The CH_2Cl_2 extracts were then evaporated to dryness in a water bath at 40° C. in a fine stream of nitrogen. Each extract was distributed between 5 ml. of distilled water and 5 ml. of ethyl acetate in order to remove traces of salts. The water layer was extracted two additional times with 5 ml. of ethyl acetate. The ethyl-acetate solutions were dried over anhydrous Na_2SO_4 and evaporated to dryness under vacuum. The dried extracts were chromatographed directly in the toluene-propylene glycol system of Zaffaroni and Burton (6). Ultraviolet-absorbing areas corresponding to

cortisol were detected in every case. They showed a characteristic reaction for α-ketols with the blue-tetrazolium reagent (7). The steroid-containing zones were eluted with $CH_3OH:CHCl_3$ (1:1) and the solvent removed by evaporation in nitrogen. Their ultraviolet absorption spectra in absolute methyl alcohol were identical with those of authentic cortisol with an absorption maximum at 241 mμ. Their concentrations were calculated on the basis of their densities at 241 mμ using the extinction coefficient of authentic cortisol in the same solvent. The deoxycortisol, the 17α-hydroxyprogesterone, and the progesterone incubations yielded respectively 0.871 mg., 2.520 mg. and 0.807 mg. of cortisol. In every case, the color developed by sulfuric acid (8) with the eluted substance was identical with that from authentic cortisol, with maxima at 238–240, 278–280, 390–393, and 475 mμ. The isolated substances were crystallized from ethyl acetate-*n*-hexane and then from acetone-*n*-hexane. The preparations from the deoxycortisol, 17α-hydroxyprogesterone and progesterone incubations melted respectively at 202–205° C., 203–206° C., and 203–206° C. On admixture of authentic cortisol, melting at 206–207° C., there was no depression of the melting point in any instance. In addition, the infrared spectra of the isolated substances were similar to that of cortisol.

The greatest conversion to cortisol, approximately 50 per cent, was obtained in the 17α-hydroxyprogesterone incubation. A section of the gland which was used appeared normal on histologic examination. The deoxycortisol and progesterone incubations yielded approximately 17 per cent and 16 per cent of cortisol, respectively. Histologic examination of sections of these glands revealed that both were invaded by carcinoma secondary to carcinoma of the mammary gland.

These studies have demonstrated the 11β-hydroxylation, 17α-hydroxylation, and 21-hydroxylation of the steroid molecule by human adrenal tissue *in vitro*.

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The Endocrine Society

THE 1957 ANNUAL MEETING

The Thirty-ninth Annual Meeting of The Endocrine Society will be held in the Hotel New Yorker, New York City, Thursday, Friday and Saturday, *May 30 and 31, and June 1, 1957.*

THE 1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should

be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

THE EIGHTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY; THE UNIVERSITY OF TEXAS POSTGRADUATE SCHOOL OF MEDICINE; AND THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE, HOUSTON, TEXAS

October 22–27, 1956

The faculty will consist of twenty-two eminent clinicians and investigators from various parts of the country in the field of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialities who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program and registration, write to *Office of the Dean, The University of Texas, Postgraduate School of Medicine, Texas Medical Center, Houston 25, Texas.* Registration is limited to 100; tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Shamrock Hilton Hotel which is adjacent to the Texas Medical Center.

(For Faculty and Program *see* the August issue of this Journal.)

The American Goiter Association

THE 1957 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Statler Hotel, New York City, on *May 28, 29 and 30, 1957*.

THE VAN METER PRIZE AWARD FOR 1957

The American Goiter Association again offers the Van Meter Prize Award of $300.00 and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association which will be held in the Hotel Statler, New York, May 28, 29 and 30, 1957, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, New York, *not later than January 15, 1957*. The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the winning essay by the author if it is possible for him to attend. The essay will be published in the annual proceedings of the Association.

BOOK REGISTER

Atlas of Exfoliative Cytology. Supplement 1. By GEORGE N. PAPANICOLAOU, M.D., PH.D. Sixteen color plates, 14 of which are devoted to the combined illustration of the cytology and corresponding histopathology in 30 cases of neoplasms involving the female genital system, urinary and male genital systems, respiratory system digestive system, exudates, and the breast. Such a comparative illustration serves to point out not only the similarities but also the dissimilarities between cells of the same type as seen in a tissue section and in a smear. Since the adoption of the cytologic method of cancer diagnosis by pathologists, these findings and the accompanying discussions, should be very helpful. Published for The Commonwealth Fund by Harvard University Press, Cambridge, Mass., 1956. Price $4.00.

Histamine. A Ciba Foundation Symposium, jointly with The Physiological Society and the British Pharmacological Society, April 6 and 7, 1955, in honor of SIR HENRY DALE; edited by G. E. W. WOLSTENHOLME and CECILIA M. O'CONNOR. This book gathers together and correlates the latest findings of internationally known authorities and is valuable as a single source of material on the rapid advances being made in histamine research today; 487 pages; illustrated; 1956. Little, Brown and Company, Boston. Price $9.00.

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